INHERITANCE IN TOMATOES[1]

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	305
Genetic relations	307
Stature and skin structure,—*Dd Pp* factors	307
Stature and flesh color,—*Dd Rr* factors	309
Stature and skin color,—*Dd Vy* factors	309
Flesh color and skin color,—*Rr Vy* factors	310
Flesh color and skin structure,—*Rr Pp* factors	312
Skin color and skin structure,—*Vy Pp* factors	313
Tests for differential fertilization or pollen-tube growth	314
Linkage groups in the tomato	315
SUMMARY	316
LITERATURE CITED	317

INTRODUCTION

In connection with my experimental work on size inheritance in the tomato (*Lycopersicum esculentum*)I have accumulated considerable genetic data on fruit characteristics and stature of plant. As these observations have an important bearing on the linkage situation in this species, it seems advisable to report them, especially since critical data have not yet been published. Because the tomato is so well adapted for a study of size inheritance it becomes imperative that the heredity of the simpler qualitative characters of this plant be thoroughly understood before the more complex problems of quantitative inheritance are attacked.

The varieties of tomatoes that I chose for work with size possessed qualitative characters, which may be described as follows:

Flesh color: Red (*R*) dominant over yellow (*r*). Gene *R* governs the formation of red needle-shaped crystals occurring within the cells of the mature fruit, while *r* produces the yellow granular color bodies. DUGGAR (1913) has named the red pigment produced by *R*, lycopersicin, replacing

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the term lycopin previously used by SCHUNCK (1903) and WILLSTÄTTER and ESCHER (1910).

Skin color: Yellow (*Y*) dominant over colorless (*y*). Gene *Y* produces a distinct, yellow pigment suffused throughout the cell walls of the epidermis of the fruit, whereas its allelomorph, *y*, produces a transparent or colorless condition in these epidermal walls.

Skin structure: Smooth (*P*) dominant over "peach" (*p*). Gene *P* is responsible for the ordinary smooth, shiny texture of the epidermis of the fruit. Such fruits also lack an appreciable amount of pubescence. Gene *p* gives a distinct pubescence on the mature fruit. The general structure of the epidermis is also changed so as to give a dull appearance to the fruit. The effect of these genes is perhaps analogous to the situation found in the nectarine and peach-tree fruits.

Stature: Tall (*D*) dominant over dwarf (*d*). In addition to the greater length of plant, the *D* factor produces a less-wrinkled type of leaf than does the *d* factor. The foliage of a dwarf plant is distinctly rugose and is much darker green in color than is the standard type.

The inheritance of each of the four pairs of characters may be explained on a single-factor basis. They are relatively easy of classification. The dominance in each case is practically complete. Notes have been taken on other characteristics, such as foliage color (dark and light green) and type of leaf, but the classification was found rather uncertain, and for this reason these characters have not been included.

Commercial varieties of tomatoes inbred one year were used as the source of material. The following is a list of such varieties together with their abbreviations and genetic formulae with respect to the four characteristics noted above:

Variety	*Constitution*
Red Cherry (RC)	*RR YY PP DD*
Bonny Best (BB)	*RR YY PP DD*
New Globe (NG)	*RR yy PP DD*
Ponderosa (P)	*RR yy PP DD*
Yellow Cherry (YC)	*rr YY PP DD*
Yellow Plum (YPl)	*rr YY PP DD*
Yellow Peach (YP)	*rr yy pp DD*
Red Peach (RP)	*RR yy pp DD*
Golden Beauty (GB)	*rr yy PP DD*
Dwarf Giant (DG)	*RR YY PP dd*

These varieties have been crossed, with the necessary precautions against contamination, in various combinations, and the F_1, F_2, F_3 and backcross generations grown both in the field and in the greenhouse.

Earlier investigations have shown that three of the four pairs of genetic factors controlling the above characteristics may be interpreted as involving a simple, monohybrid situation (CRAIG 1907; GILBERT 1912; HALSTED 1905; HEDRICK and BOOTH 1907). Apparently but little critical data have been published concerning the inheritance of the "peach" type of fruit (PRICE and DRINKARD 1908), but this character I find to be a simple recessive to the smooth or non-pubescent type, characteristic of most of the commercial varieties.

As far as I have been able to ascertain, the linkage relations of these factors have not been investigated to any extent. JONES (1917) has listed a few cases of linked inheritance in the tomato, from data reported by other investigators, but in these linkages one of the characters involved has been of a quantitative nature, the mode of heredity of which is still unknown.

GENETIC RELATIONS

Stature and skin structure,—Dd Pp factors

Complete linkage exists between the *Dd* (tallness *versus* dwarfness) and the *Pp* (smooth *versus* peach skin) pairs of factors. This I observed clearly in the F_2 generation of the cross Dwarf Giant (*dd PP*) × Yellow Peach (*DD pp*). Seed from the F_1 generation, which was tall in stature and bore smooth or non-pubescent fruit, gave in the F_2 the following distribution among a total of 209 plants: 101 tall smooth : 56 tall peach : 52 dwarf smooth : 0 dwarf peach. No double-recessive plants (*dd pp*) appeared. A few of the dwarf F_2 plants were tested in the F_3 generation and all bred true to the smooth type of fruit as well as to dwarfness, indicating that the dwarf-peach combination does not appear at all.[2]

Such data obviously are characteristic of complete linkage. The approximation to the 2 : 1 : 1 ratio typical of such a situation is extremely

[2] In one later F_3 progeny from an open-pollinated dwarf smooth F_2 plant, two distinct peach (pubescent) dwarfs appeared among five plants. On the complete-linkage hypothesis these could scarcely have occurred by pollen contamination, since the mother plants were presumably homozygous *dP.dP*. The only obvious explanation is that of mutation from *dP.dP* to *dP.dp* in the F_2 plant, whereby the peach type would appear in F_3 as a recessive segregate. That such mutations of pubescence occur is not uncommon, especially in the case of the nectarine and the peach-tree fruits. I have also observed in the tomato that in F_2 and F_3 generations involving the smooth and peach types, considerable variation in the degree of pubescence occurs. In some cases I have seen in the same fruit-cluster of an F_2 or F_3 peach type, a relatively smooth fruit at maturity. Some of these variations are now being investigated.

The quadruple recessive type, *rryyppdd*, which I have just developed from the plants noted above, has been crossed with the tetrahybrid condition, *RrYyPpDd*, and should give some critical data on the *P-D* linkage.

close, the deviations from the calculated proportion of 105 : 52 : 52 being only 4 : 4 : 0, respectively.

An interesting verification of the complete-linkage theory was afforded by some F_3 seedling progeny tests. Ten F_3 progenies from tall-peach type F_2 plants all bred true for the tall character. Of ten F_3 progenies grown from tall-smooth type F_2 plants, none bred true for tallness, all segregated for dwarfs. In other words, one can predict which of the F_3 progenies, arising from the dominant tall F_2 plants, will breed true for tallness by the type of skin structure of the mother plants. The F_3 progenies from dwarf-smooth F_2 plants, ten in number, all bred true for dwarfness. These facts are in full accord with the expectation if *D* and *P* are considered as being completely linked in inheritance.

This situation is perhaps known in the commercial world, since there are no dwarf varieties recorded with "peach" skin. GROTH (1910) noted this phenomenon in the days before genetic linkages were understood.

In any case of complete linkage it becomes of interest to speculate whether the situation really is one in which two different pairs of genetic factors are actually correlated in inheritance because they are borne on the same chromosome, or whether there is fundamentally only one pair of factors with a third allelomorph concerned. On the basis of complete linkage, the following three genotypes only are expected in the F_2 generation:

1 *Dp* . *Dp*,—tall peach
2 *Dp* . *dP*,—tall smooth
1 *dP* . *dP*,—dwarf smooth

This situation accounts for the experimental facts very well.

If we could conceive of some physiological correlation between factors for stature and those for structure of skin, we might assume that multiple allelomorphs were concerned. That there may be some apparent relation here is not altogether inconceivable if we think in general terms. The "peach" factor is one that permits the epidermal hairs to persist on the fruit until mature. At least this is its most obvious effect, although the actual structure and appearance of the epidermis are also modified. In "smooth" fruits the epidermal hairs are apparently formed but drop or fail to develop in the earlier stages of fruit growth. It is conceivable that this same effect of "persistence" might be related to the tendency of the plant to persist in growing until it eventually develops into the tall type of plant. However, the fact that we have true-breeding varieties that normally are tall and *smooth-fruited* would seem to invalidate this physiological-correlation hypothesis. Also, there appears to be no obvious

reason for any physiological incompatibility between dwarfness and the peach type of fruit.

Accordingly, complete linkage of two different factor pairs seems to present the simpler and more reasonable explanation of the inheritance of these characters.

Stature and flesh color,—Dd Rr factors

These factors are inherited independently of each other. The F_2 data of the crosses, Dwarf Giant (*dd RR*) × Yellow Peach (*DD rr*), and Dwarf Giant × Yellow Cherry (*DD rr*), demonstrating this, have been arranged in table 1. There is not sufficient deviation from the 9 : 3 : 3 : 1 ratio in

TABLE 1

F_2 data involving factors Dd Rr from the parental combinations Dr dR.

CROSSES	TALL RED *DR*	TALL YELLOW *Dr*	DWARF RED *dR*	DWARF YELLOW *dr*
Dwarf Giant × Yellow Peach	121	36	36	16
Dwarf Giant × Yellow Cherry	48	18	9	2
Total	169	54	45	18
Theoretical	*160.9*	*53.6*	*53.6*	*17.9*

Deviation from 10 : 6 ratio = 8.25 ± 5.52

these hybrid generations to justify any suspicion of linkage. When the parental classes (*Dr* and *dR*) are grouped against the non-parental classes (187 : 99) the conclusion that *D* and *R* are independently inherited becomes obvious, since the deviation from the 10 : 6 ratio in this case is only about 1.5 times as large as the probable error.

Stature and skin color,—Dd Yy factors

Dwarfness is also inherited independently of skin color. Proof for this is afforded by the F_2 generation of the cross, Dwarf Giant (*dd YY*) × Yellow Peach (*DD yy*). From a total of 209 F_2 plants the following results were obtained:

123 tall yellow-skin (*DY*) : 34 tall colorless-skin (*Dy*) : 37 dwarf yellow-skin (*dY*) : 15 dwarf colorless-skin (*dy*).

The theoretical expectancy on a 9 : 3 : 3 : 1 basis is 118 : 39 : 39 : 13. Obviously, the agreement is so close that factors *D* and *Y* must be independently inherited.

Flesh color and skin color,—Rr Yy factors

These factors are responsible for the actual color of the tomato fruit (HURST 1906). Their four different combinations produce the following well-known colors:

RY, orange red (due to red flesh showing through yellow skin)
Ry, red (the pink or purple of commerce)
rY, dark yellow
ry, pale yellow

I have experienced no real difficulty in classifying mature tomato fruit colors on this basis, despite the fact that occasionally a yellow-fleshed fruit will show a faint red blush at the distal end.

Data from F_2 and backcross generations involving both the "coupling" and "repulsion" phases of linkage are available and have been arranged in tables 2 and 3.

TABLE 2

Fruit color in F_2 generations of various tomato crosses involving flesh and skin colors.

CROSSES	*RY*	*Ry*	*rY*	*ry*	DEVIATION FROM 10 : 6 RATIO
F_2 of *RR YY* × *rr yy*					
Red Cherry × Golden Beauty	34	7	6	3	
Golden Beauty × Red Cherry	50	18	6	4	
Yellow Peach × Bonny Best	51	14	11	3	
Bonny Best × Yellow Peach	41	12	10	6	
Dwarf Giant × Yellow Peach	118	39	42	10	
Total	294	90	75	26	17.0±7.2
Expected	*273*	*91*	*91*	*30*	
F_2 of *RR yy* × *rr YY*					
New Globe × Yellow Cherry	14	8	0	3	
Yellow Cherry × New Globe	39	10	10	1	
Ponderosa × Yellow Plum	31	7	10	4	
Total	84	25	20	8	6.0±3.8
Expected	*77.0*	*25.7*	*25.7*	*8.6*	

In table 2, which gives the F_2 generations of five different crosses, there is an approximation to a dihybrid distribution with independent inheritance. Where *R* and *Y* were contributed by the same parent, there resulted 294 orange-red : 90 red : 75 yellow : 26 pale yellow, when 273 : 91 : 91 : 30, respectively, were expected. Grouping the parental classes (*RY* and *ry*) against the non-parental (*Ry* and *rY*), there results 320 : 165. This

deviates from the 10 : 6 ratio of independent inheritance by 17 individuals, a deviation which is 2.4 times larger than its probable error, and might suggest linked inheritance. However, the excess of red-fleshed fruits is responsible in some measure for the large deviation.

Where *R* and *y* were contributed by the same parent the F_2 distribution was 84 : 25 : 20 : 8, when 77 : 26 : 26 : 9, approximately, were to be expected. This gives 92 non-parental and 45 parental combinations where 86 and 51, respectively, are to be expected. In this case the deviation of 6 is in the wrong direction to indicate any linkage.

TABLE 3

Fruit color in backcross generations of various tomato crosses involving flesh and skin colors.

CROSSES*	*RY*	*Ry*	*rY*	*ry*	PERCENT CROSSING OVER
Coupling phase (*RY* × *ry*)					
F_1 (RC × GB) × YP	21	9	5	9	
F_1 (GB × RC) × YP	9	13	10	8	
YP × F_1 (GB × RC)	13	13	8	6	
F_1 (GB × RC) × GB	9	3	9	9	
GB × F_1 (GB × RC)	11	9	4	6	
F_1 (YP × BB) × YP	9	9	19	13	
YP × F_1 (YP × BB)	9	11	12	18	
F_1 (YP × BB) × GB	7	9	6	6	
F_1 (BB × YP) × YP	12	8	9	11	
YP × F_1 (BB × YP)	12	9	11	8	
Total	112	93	93	94	47.4±1.7
Expected	*98*	*98*	*98*	*98*	
Repulsion phase (*Ry* × *rY*)					
F_1 (NG × YC) × YP	7	14	7	12	
F_1 (NG × YC) × GB	4	5	9	2	
F_1 (YC × NG) × YP	12	14	8	5	
F_1 (YC × NG) × GB	12	5	6	7	
Total	35	38	30	26	47.3±3.0
Expected	*32*	*32*	*32*	*32*	

* Abbreviations in Roman capital letters are variety names, RC being Red Cherry, etc.

The backcross generations of the same crosses, listed in table 3, offer some indications of a very loose linkage between *R* and *Y*. Both the "coupling" and "repulsion" phases of the situation show this. In one case there is 47.4±1.7 percent and in the other 47.3±3.0 percent crossing over. When, however, these crossover percentages are compared with their

probable errors, the deviations from 50 percent crossing over, characteristic of independent inheritance, fall within the limits of error for inheritance upon an independent basis. To test the actuality of such a loose linkage, much larger numbers are necessary. Undoubtedly, many investigators have such data on hand and it is hoped that they will report them in the near future. In view of the absence of linkage in one of the F_2 generations, however, it is reasonable to suppose that factors *R* and *Y* will be found to be inherited independently of each other.

Flesh color and skin structure,—Rr Pp factors

The "peach" type of skin, which is completely linked with stature of plant, is apparently due to the effect of a single recessive gene, and this factor seems to be inherited independently of red and yellow flesh color governed by the *Rr* factor pair. Data supporting this conclusion are provided by F_2 and backcross generations of the crosses shown in tables 4 and 5.

TABLE 4

F_2 data involving factors Rr Pp, the parental combinations being RP and rp.

CROSSES	RED SMOOTH *RP*	RED PEACH *Rp*	YELLOW SMOOTH *rP*	YELLOW PEACH *rp*
Bonny Best × Yellow Peach	84	34	24	6
Dwarf Giant × Yellow Peach	111	46	43	9
Total	195	80	67	15
Expected	*201*	*67*	*67*	*22*

Deviation from 10 : 6 ratio = 13.0 ± 6.2

TABLE 5

Backcross data involving Rr Pp factors, the parental combinations being RP and rp.

CROSSES	*RP*	*Rp*	*rP*	*rp*
F_1 (BB × YP) × YP	19	19	26	26
YP × F_1 (BB × YP)	20	21	26	22
Total	39	40	52	48
Expected	*44.75*	*44.75*	*44.75*	*44.75*

Crossing-over percentage = 51.4 ± 2.5

In table 4 the F_2 generations derived from the crosses, Bonny Best × Yellow Peach and Dwarf Giant × Yellow Peach, both crosses involving *RP* and *rp* as parental combinations, approximate fairly closely the

9 : 3 : 3 : 1 ratio of independent inheritance. The parental classes total 210 and the non-parental 147, where 223 and 134, respectively, are to be expected. This shows a deviation of 13, which is only 2.1 times as large as its probable error.

The backcross data of the Bonny Best × Yellow Peach cross appearing in table 5 show conclusively that no linkage is present. In this case the crossover percentage is calculated as 51.4±2.5 percent, indicating independent inheritance between *R* and *P*. Such independent inheritance must follow, of course, since *P* and *D* are completely linked, and *D* and *R* have been shown in a preceding section to be independent.

Skin color and skin structure,—Yy Pp factors

The "peach" characteristic is also transmitted independently of the skin-color factor pair, *Yy*. Here again F_2 and backcross data from the same crosses are available as shown in tables 6 and 7. Both of these distributions are, without doubt, in excellent agreement with the F_2, 9 : 3 : 3 : 1, and the backcross, 1 : 1 : 1 : 1, ratios. Accordingly, the genes *Y* and *P*

TABLE 6

F_2 data involving Yy Pp factors, the parental combinations being YP and yp.

CROSSES	YELLOW-SKIN SMOOTH *YP*	YELLOW-SKIN PEACH *Yp*	COLORLESS-SKIN SMOOTH *yP*	COLORLESS-SKIN PEACH *yp*
Bonny Best × Yellow Peach	85	28	23	12
Dwarf Giant × Yellow Peach	118	42	36	13
Total	203	70	59	25
Expected	*201*	*67*	*67*	*22*

Deviation from 10 : 6 ratio = 5.0±6.2

TABLE 7

Backcross data involving Yy Pp factors, the parental combinations being YP and yp.

CROSSES	*YP*	*Yp*	*yP*	*yp*
F_1 (BB × YP) × YP	21	28	24	17
YP × F_1 (BB × YP)	23	20	23	23
Total	44	48	47	40
Expected	*44.75*	*44.75*	*44.75*	*44.75*

Crossing-over percentage = 53.1±2.5

are not linked. This cross is a verification of the independent inheritance between *Y* and *D*, a situation which must follow if *D* and *P* are completely linked.

TESTS FOR DIFFERENTIAL FERTILIZATION OR POLLEN-TUBE GROWTH

A fairly large series of backcrosses, made reciprocally, afford some information on whether or not there is in the tomato differential fertilization or pollen-tube growth. My data on this point involve the flesh colors,

TABLE 8

Summary of backcross data for comparison of reciprocal backcrosses testing differential fertilization or pollen-tube growth involving the genes Rr, Yy and Pp.

BACKCROSSES	*R*	*r*	*Y*	*y*	*P*	*p*
F_1 as ♀ parent						
(YC × BB) × YC	49	46				
(YC × BB) × YP	25	15				
(GB × RC) × YC	23	37				
(RC × GB) × YP	30	14	26	18		
(GB × RC) × YP	22	18	19	21		
(GB × RC) × GB	12	18	18	12		
(YP × BB) × YP	18	32	28	22	30	20
(BB × YP) × YP	20	20	21	19	15	25
(YP × BB) × GB	16	12	13	15		
(NG × YC) × YP	21	19	14	26		
(YC × NG) × YP	26	13	20	19		
(YC × NG) × GB	17	13	18	12		
(NG × YC) × GB	9	11	13	7		
Total observed	288	268	190	171	45	45
Expected	*278*	*278*	*180.5*	*180.5*	*45*	*45*
Percent observed	51.8	48.2	52.6	47.4	50.0	50.0
F_1 as ♂ parent						
YC × (YC × BB)	38	30				
YC × (GB × RC)	30	30				
YP × (GB × RC)	26	14	21	19		
GB × (GB × RC)	20	10	15	15		
YP × (YP × BB)	20	30	21	29	24	25
YP × (BB × YP)	21	19	23	17	22	18
Total observed	155	133	80	80	46	43
Expected	*144*	*144*	*80*	*80*	*44.5*	*44.5*
Percent observed	53.8	46.2	50.0	50.0	51.7	48.3

R and *r*, the skin colors, *Y* and *y*, and the skin-structure genes, *P* and *p*. By comparing the observed results in the backcross progenies when the

F_1 is used as the pistillate parent with results when it is used as a staminate parent, it is possible to detect any excess of one character over another (see table 8).

With respect to the flesh color genes, *Rr*, it may be seen in table 8 that there is an excess of red-fruited plants in both series of backcrosses. When the F_1 is used as the pollen parent there is 2.0 percent more red fruits than when it is used as the ovule parent. This suggests that the pollen-tubes bearing the *R* gene are somewhat more effective in fertilization than those carrying the yellow *r* factor. However, the deviation (11.0 ± 5.7) in this case is only twice as large as the probable error, and accordingly affords no indisputable proof of such differential action.

With none of the other factors is there any significant difference in the two series of backcrosses. The numbers involved are somewhat meager, and reliance could not be placed on them save where unusually large differences existed.

LINKAGE GROUPS IN THE TOMATO

Heretofore, no critical data have been reported on genetic linkage in the tomato. It is true that HEDRICK and BOOTH (1907) suggested a correlation between fruit shape (pyriform) and the *Dd* factor pair, and JONES (1917) concurs in this idea. As far as I am aware, however, no one has yet proven that fruit shape in tomatoes can be resolved into simple genetic factors. My experience is that the heredity of fruit shape is relatively complex, and has as yet not been analyzed into its component parts. To compute a crossing-over percentage for such a linkage is accordingly somewhat premature.

The same criticism applies to the linkage first reported by PRICE and DRINKARD (1908) and listed by JONES (1917), involving foliage color and number of locules in the fruit. Despite the frequent assertions or implications that locule number is dependent upon a single pair of genes, I venture to suggest that this is not the case. In my work, at least, there are several different factors concerned. Because of the partial dominance of the two-loculed condition and the resulting skew curve in the F_2 generation, there is a temptation to interpret this quantitative data on a single-factor basis. As far as I know no critical F_3 tests have ever been reported to substantiate the claim of a one-factor basis for locule number.

CRANE (1915) reports a similar situation with respect to a correlation involving quantitative characters. He suggests that a linkage exists between general fruit shape (not-pyriform) and certain types of inflorescence.

His data are meager, however, and he does not stress this relation, nor to my knowledge has it been followed up.

In 1924, I reported a case in which the *Yy* pair of factors was apparently linked with a certain major factor for total size or weight of fruit. Since then I have verified this report with other crosses and have evidence to prove that this is a legitimate genetic linkage and not a physiological correlation. The size factors involved in this linkage are a partially dominant factor for small size that is characteristic of the Cherry type of fruit, contrasted with a factor for large size contributed by the Golden Beauty variety. The *Y* gene is not necessarily linked with all size factors, but rather with certain ones characteristic of definite, pure varieties. In fact, I have crosses in which the *Yy* factors are most certainly not linked with size factors brought in by some of the larger-fruited sorts. In some of these crosses the *Rr* factors show a decided linkage with size factors. Data on these linkages are forthcoming in a later publication.

If we dismiss temporarily these cases of linkage, because at least some of the characters involved are as yet too complex to actually isolate the quantitative gene concerned, we are forced to the conclusion that linkage groups in the tomato are as yet rare. The complete linkage existing between the *Dd* and *Pp* factors seems to be a legitimate case.

Accordingly, it is worth recording that the following genetic factors in the tomato form the basis for three different linkage groups:

Group 1. *Dd* and *Pp*
Group 2. *Rr*
Group 3. *Yy*

All the necessary tests have been made to demonstrate the truth of this situation with the exception of a rare possibility that *R* and *Y* are loosely linked. When we consider that the tomato has been reported as possessing twelve pairs of chromosomes it is not surprising, of course, that more linkages have not been discovered.

SUMMARY

1. Complete linkage exists between the *Dd* pair of genes controlling growth habit (tall *versus* dwarf) and the *Pp* pair responsible for skin structure (smooth *versus* "peach" type of fruit).

2. Genes *Rr* involved in flesh color (red *versus* yellow) are inherited independently of both *Dd* and *Pp*.

3. The factors *Yy* controlling skin color are undeniably independent of *Dd* and *Pp* and very likely of *Rr*.

4. These series of interrelations permit the establishment of three provisional linkage groups in the tomato.

5. Backcross data afforded some evidence of a slight differential fertilization or pollen-tube growth involving the genes *R* and *r*.

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INHERITANCE OF PALE GREEN SEEDLINGS IN MAIZE[1]*

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	318
Review of literature	318
Description of material used	319
Description of different pale-green types	322
Intercrosses between different pale-green types	323
Linkage relations	325
Linkage tests with pale-green 2	326
Linkage between $P_{g2}p_{g2}$ and *Dd*	326
Test between $P_{g2}p_{g2}$ and W_xw_x	326
Tests between $P_{g2}p_{g2}$ and S_hs_h, *Cc*, *Rr*, S_us_u, *Yy*	326
Linkage tests with pale-green 3	327
Linkage between $P_{g3}p_{g3}$ and B_nb_n	327
Linkage tests with pale-green 4	327
DISCUSSION	329
SUMMARY	331
LITERATURE CITED	331
APPENDIX—Tables 5 to 26	334

INTRODUCTION

One of the many chlorophyll abnormalities in maize is the pale green seedling which can frequently be observed upon selfing of practically any commercial variety of maize. If the search for chlorophyll abnormalities is made on a large scale different intensities of pale green seedlings may easily be found. The results of the investigations presented in this paper show that several of the phenotypically different pale green seedlings are not due to the difference in the expression of one gene, but are determined by different and independent genes.

REVIEW OF LITERATURE

A pale green type in Mirabilis was described by CORRENS (1902, 1903) as early as 1902, even though the data as to its inheritance were not presented until 1909 (CORRENS 1909). Later, BAUR (1907, 1908) analyzed genetically an *aurea* pale green type in Antirrhinum, and PRICE and DRINKARD (1908) studied the inheritance of a pale green variety of Lycopersicum (tomato). Subsequently, Mendelian pale green types having

* The GALTON AND MENDEL MEMORIAL FUND has met in part the cost of setting the tables in this paper.

[1] Investigations reported in this paper were begun at the Department of Plant Breeding of CORNELL UNIVERSITY, Ithaca, New York, where the majority of tests were completed.

different chlorophyll content have been analyzed, as far as known to the author, in the following plants: Urtica (CORRENS 1909, 1922), Antirrhinum (BAUR 1910), Aquilegia (BAUR 1910), Pelargonium (BAUR 1911, p. 166, NOACK 1924), Melandrium (SHULL 1913), Secale (NILSSON-EHLE 1913), *Capsella bursa-pastoris* (CORRENS 1919 a), Arabis (CORRENS 1919 b), Nicotiana (ALLARD 1919, KAJANUS 1924), Allium (RASMUSON 1920), Ipomea (CORRENS 1920), Mercurialis (CORRENS 1920), Papaver (RASMUSON 1920), Tropaeolum (CORRENS 1920), Festuca (KAJANUS 1921), Avena (ÅKERMAN 1922), Hordeum (NILSSON-EHLE 1922, HALLQVIST 1924), and Polemonium (DAHLGREN 1924).

The first pale green type in maize was described by EMERSON (1912 a) and was named golden, because it "resembled in color the so-called "golden" varieties of various ornamental shrubs." A detailed description, together with the genetic evidences as to the relation between that type and certain other characters affecting the development of chlorophyll, was given by LINDSTROM (1918). Golden is a simple Mendelian recessive to green. Golden plants are normal green in appearance in the seedling stage. With age, however, they partially lose their chlorophyll and become golden yellow when fully developed.

BRUNSON (1924) described the second pale green type in maize, called, "pale green 1" (P_{g1} p_{g1}), which, in general, resembles the types described in this paper. Pale green 1 is a lethal seedling character having about 15 percent of the normal chlorophyll and about 50 percent of the normal xanthophyll content.

TRAJKOVICH (1924) presented the evidence on the inheritance of another pale green type in maize called *xantha* (X_n x_n), which, in spite of having only about 10 percent as much chlorophyll as a normal green plant, can live to maturity if grown under favorable conditions.

DESCRIPTION OF MATERIAL USED

Sufficient tests were made to warrant the description of five pale green types with which the investigation was begun in the winter of 1919. Four of them will be named pale green 2, 3, 4 and 5 (P_{g2} p_{g2}, P_{g3} p_{g3}, P_{g4} p_{g4}, $P_{g5}p_{g5}$), and the fifth one, which resembles closely *xantha* described by Mrs. TRAJKOVICH (1924), will be named *xantha* 2 (X_{n2} x_{n2}). The name *xantha* 1 (X_{n1} x_{n1}) is applied to *xantha* already described. Different types are distinguished by numbers rather than by separate names mainly because numbering simplifies the nomenclature in maize, which is becoming more and more complicated by the progress of the genetical analysis of that plant.

An estimate of the chlorophyll content was made for all of the pale green types. For that purpose, a weighed amount of young leaves was ground, the pigment extracted with ethyl alcohol and the chlorophyll separated from xanthophyll and carotin with petrol-ether. The same proportions of leaves and solvents were used in extracting the chlorophyll from green and pale green types; the percent of chlorophyll and xanthophyll in pale green as compared with normally green types was computed by adding to the solution extracted from normally green leaves enough solvent to make the color of this solution match that of the pale green leaves. Pale green seedlings were always compared with the normals from the same family. In several instances chlorophyll content was determined several times during the investigations and in these cases the highest and lowest values are given in table 1. Each determination is an average of at least three determinations made from the same material.

TABLE 1

Chlorophyll content of different pale green types found in maize.

TYPE	PERCENTAGE OF CHLOROPHYLL	PERCENTAGE OF XANTHOPHYLL	DESCRIBED BY
p_{g1}	53	Normal	
p_{g1}	28.2—30.7	Normal	
p_{g1}	38.5—50.5	Normal	
p_{g1}	71.5—83.3	Normal	
x_{n1}	10	Normal	
Types already described			
p_{g1}	15	50	BRUNSON 1924*
x_{n1}	10.7	Normal	TRAJKOVICH 1924*
g Upper leaves	50	Normal	EMERSON 1912 a
g Lower leaves	8.7	Normal	LINDSTROM 1918

* Chlorophyll estimate by BRUNSON and Mrs. TRAJKOVICH, respectively, was made according to the same method as that used by the author.

The chlorophyll content of pale green and *xantha* types was determined in the early seedling stage at the age of two to four weeks, depending on the temperature at which the seedlings were grown. Since golden plants are green in early life, becoming yellow only when older, the chlorophyll content was determined at the tasseling stage, when the upper leaves had just begun to lose their chlorophyll and the lower ones were already golden yellow.

As can be seen from table 1, pale green types differed appreciably in the amount of chlorophyll they contained. Xanthophyll content, except in the case of p_{g1} p_{g1}, was found to be practically normal. It ought to be mentioned, however, that while no difficulty was experienced in matching the color of green solutions, great difficulty was encountered in trying to match exactly the yellow color in diluted solutions. Very slight, if any, difference in color was observed between the xanthophyll extracts from normal and pale green types and the conclusion was reached in each case, that the pale green seedlings have a normal content of xanthophyll and carotin. *Xantha* 1 and *xantha* 2 seedlings had practically the same amount of chlorophyll.

Individual variations in color between the seedlings of the same type were not great, but still they were appreciable, especially so in the case of pale green 2. These variations made the determination of color according to RIDGWAY'S (1912) Color Standards uncertain and unreliable.

A common characteristic of pale green seedlings is that they are lethal, or nearly lethal. Pale green 5, for example, which has between 70 and 80 percent of the normal amount of chlorophyll, does not live much longer than white seedlings, which do not have any chlorophyll at all. At the age of about three to four weeks the leaves of pale green seedlings appear as if water-soaked. In a few days more they wilt and die without losing their chlorophyll content. Closer examination of the dying plants showed that they had a well developed root system but that the roots were practically dead, just as was the case with albinos. This may indicate that the chlorophyll in the pale green seedlings does not develop to the stage in which it is capable of photosynthesis and that the roots and leaves die because of lack of food. It is highly improbable that the lack of chlorophyll is the cause of death of pale green seedlings. As shown by TRAJKOVICH (1924), if conditions are favorable, a maize plant may live and produce a tassel and seed, though it has only about ten percent of the normal amount of chlorophyll.

In an attempt to keep the pale green seedlings alive, they were grown in soils of different fertility, under lights of different duration and intensity and under different temperatures. All attempts were unsuccessful in the case of P_{g3} p_{g3}, P_{g4} p_{g4} and P_{g5} p_{g5}, which always died after a certain stage of development had been reached. In the case of P_{g2} p_{g2}, under favorable conditions a small percentage of seedlings passed the critical stage and grew to maturity. The seedlings turned green and did not differ either in color or in vigor from other normally green plants in the later stage of their life. Different lines of $P_{g:}$ $p_{g:}$ differed in the number

of pale green seedlings which became green under similar conditions, which may indicate the presence of a modifying factor or factors which stimulate the development of chlorophyll.

DESCRIPTION OF DIFFERENT PALE GREEN TYPES

Pale green 2 (p_{g2} p_{g2})

Pale green 2 (P_{g2} p_{g2}) has about 53 percent of the normal chlorophyll and practically normal xanthophyll content. It is very variable in color with extreme variations which can hardly be distinguished from normal green seedlings. This renders classification rather difficult, especially if the seedlings are grown in warm surroundings. When grown at about 15 to 18 degrees centigrade, p_{g2} p_{g2} can easily be distinguished from green seedlings. Otherwise, the seedlings have to be closely observed during their growth, and classification made several times. Most of the pale green seedlings die in the seedling stage, but if the conditions are favorable for growth, a small percentage of them may live through the critical stage and reach maturity. Mature plants are green and can not be distinguished from normally green plants.

It was found that p_{g2} p_{g2} is linked with the factors for dwarf (*Dd*), the percentage of crossing over being about 32.

A selfed ear, number 8946-4, taken from Professor EMERSON'S cultures, gave the original material for pale green 2.

Pale green 3 (p_{g3} p_{g3})

An analysis made in 1922 showed that p_{g3} p_{g3} has about 28 percent of the normal chlorophyll content, and another one made in 1923 showed about 30.7 percent. The xanthophyll content in both cases was not different from that in normal green seedlings. Pale green 3 is of a very distinct type and no difficulty was experienced in separating it from green seedlings. No attempt to grow p_{g3} p_{g3} to maturity has been successful. Under all conditions which were tried, pale green seedlings died in the three- or four-leaf stage.

It was found that P_{g3} p_{g3} is linked with brown aleurone color ($B_n b_n$).

Pale green 3 was first observed in the progenies grown from a selfed ear, number 46-1, taken from Doctor E. G. ANDERSON'S cultures.

Pale green 4 (p_{g4} p_{g4})

This type is slightly greener than p_{g3} p_{g3}, having, according to the analysis made in January 1922, 50.5 percent, according to the analysis

of April of the same year 44.5 percent, and according to the analysis of February 1923, 38.5 percent, of normal content of chlorophyll. In each of the analyses progenies from different plants were used, which may account for the differences in the results. These differences, however, may be due to the different stage of development found at the time when the analysis was made. As far as tests were made, $p_{g4}p_{g4}$ acted always as a lethal character, all plants dying in the early seedling stage.

Pale green 4 was found in the progenies from ear number 8935-3 from Professor EMERSON'S cultures.

Pale green 5 (p_5p_5)

In the early seedling stage $p_{g5}p_{g5}$ can hardly be distinguished from the green seedlings. According to the analysis made in the winter of 1922 it had 83.3 percent, and according to that of 1923 it had 71.5 percent, of normal chlorophyll content. The difference between pale green and green seedlings is so small that it is not easy to detect it. When seedlings grow older, however, pale green ones begin to appear water-soaked and invariably die before they reach the age of four or five weeks. Seedlings were grown under various environmental conditions, but under none of them did pale green seedlings survive.

Pale green 5 came from ear number 5533-8 of Professor EMERSON'S cultures.

Xantha 2 ($x_{n2}x_{n2}$)

According to chlorophyll analysis and appearance, *xantha* 2 looks like *xantha* 1 described by Mrs. TRAJKOVICH (1924).

No attempt was made to grow *xantha* 2 plants to maturity, but the tests so far indicate that the plants live longer than other pale green seedlings, which makes it probable that *xantha* 2, under favorable conditions, would grow to maturity as *xantha* 1 does.

Xantha 2 was found among the progenies of a selfed ear belonging to the variety of dent maize called Falconer, the seeds of which were obtained from Mr. O. H. WILL of Bismarck, North Dakota.

INTERCROSSES BETWEEN DIFFERENT PALE GREEN TYPES

Since the majority of the pale green types are lethal it was not possible to make the intercrosses between them by using homozygous pale green plants, but it was necessary to make crosses with the green plants from families which throw pale greens. In such families two-thirds of the plants should be heterozygous for pale green. Whenever possible, the same

method of intercrossing was used as that used in the work with white seedlings (DEMEREC 1923); that is, if lines to be intercrossed differed in endosperm, or aleurone color, or texture, a plant from a line having the dominant character was selfed, and a plant from the line with the recessive character was pollinated with a mixture of its own pollen and that from the selfed plant. On the plant pollinated by the mixture of pollen it was possible by xenia to distinguish seeds which were the result of self-pollination from those that come from the cross. In that way it was possible to determine if the plants used in intercrosses were heterozygous for pale green, because both parent plants were selfed at the time the cross was made.

TABLE 2

Summary of data from intercrosses between different pale green types. For detailed F_2 data see tables 5 to 12. The F_1 in each cross was green.

		p_{g2}		p_{g4}		p_{g5}		x_{n1}	
		Normal	Pale	Normal	Pale	Normal	Pale	Normal	Pale
f_{g2}	F_2 Observed	1234	893	1567	1281	353	268	348	279
	Calculated 9 : 7	*1196*	*931*	*1602*	*1246*	*349.3*	*271.7*	*352.7*	*274.3*
	Difference	34.00 ± 15.45		35.00 ± 17.85		3.70 ± 8.34		4.70 ± 8.38	
p_{g3}	F_2 Observed			371	305	73	55	163	109
	Calculated 9 : 7			*380.25*	*295.75*	*72*	*56*	*153*	*119*
	Difference			9.25 ± 8.70		1.00 ± 3.79		10.00 ± 5.52	
p_{g4}	F_2 Observed					725	541	F_1 green	
	Calculated 9 : 7					*712*	*554*	F_2 not tested	
	Difference					13.00 ± 11.85			
p_{g5}								F_1 green	
								F_2 not tested	
x_{n1}	F_2 Observed							322	259
	Calculated 9 : 7							*326.8*	*254.2*
	Difference							4.80 ± 8.07	

Table 2 gives a summary of the results from intercrosses between different pale green types, from which it can be seen that the tests with $p_{g2}p_{g2}$ $p_{g3}p_{g3}$, $p_{g4}p_{g4}$, $p_{g5}p_{g5}$ and $x_{n2}x_{n2}$ are complete enough to warrant the conclusion that these pale green types are determined by different genetic factors. The F_1 generation in all eleven possible intercrosses was green, nine crosses being carried to F_2 where a dihybrid 9 : 7 ratio was observed in each case. The crosses between $p_{g4}p_{g4}$, $p_{g5}p_{g5}$, and $x_{n2}x_{n2}$ were

not carried farther than F_1, but green F_1 generations supported by the phenotypical difference between those types, offers a firm ground for the conclusion that they are genetically different.

None of the pale green types described in this paper have been intercrossed with $p_{g1}p_{g1}$ (BRUNSON 1924), but from indirect evidence it may be inferred that they are genetically different. As shown by BRUNSON, $P_{g1}p_{g1}$ is linked with the *Rr* factors, the percentage of crossing over between them being 23.3. The data presented in tables 16, 21, 22, and 23 in the appendix and discussed on pages 326-328 show conclusively that $P_{g2}p_{g2}$ and $P_{g4}p_{g4}$ are inherited independently from *Rr* and consequently they must be determined by a pair of genes different from $P_{g1}p_{g1}$. The supposition that $P_{g3}p_{g3}$ is not linked with *Rr* is supported by the fact that it was found to be very closely linked with brown aleurone (B_n b_n), which is known to be independent from *Rr* (KVAKAN 1924). Since B_nb_n and *Rr* are independent in inheritance, $P_{g3}p_{g3}$ and *Rr* could not possibly be linked together with a percentage of crossing over of 23.3, and therefore $p_{g3}p_{g3}$ and $p_{g1}p_{g1}$ can not be determined by the same pair of genes. The phenotypical difference between $p_{g1}p_{g1}$ and $p_{g5}p_{g5}$ is so great as to make it highly probable that these two pale green types are genetically different. Pale green 1 contains approximately 15 percent as much chlorophyll and 50 percent as much xanthophyll as normal green seedlings, and $p_{g5}p_{g5}$ has about 80 percent of normal chlorophyll and practically normal xanthophyll content.

The 9 : 7 ratio of green to pale green seedlings which is observed in the F_2 generation is a dihybrid ratio in which three pale green classes are combined. It should be a $9P_g : 3p_{gx} : 3p_{gy} : 1p_{gx}p_{gy}$ ratio. The distinction, however, between different pale green types, when grown in the same family, is very uncertain and no attempt was made to separate them. A double recessive class also could not be detected with certainty. By analogy with virescent seedlings (DEMEREC 1924) and other genetic material, it might be expected that double recessives would have less chlorophyll than either of the parents, but the apparent difference in color was so slight that the classification was not dependable.

LINKAGE RELATIONS

Linkage tests were made between certain pale green types and several genetically known characters in maize, the descriptions of which are as follows:

Aa, Anthocyan pigment. A factor pair for pigmentation of aleurone, pericarp and plant (EMERSON 1918, 1921).

B_nb_n, Brown aleurone (KVAKAN 1924).

Cc, A factor pair for aleurone color. Complementary to *Aa* and *Rr*. (EMERSON 1918).

Rr, A factor pair for aleurone color. Complementary to *Cc*. (EAST and HAYES 1911, EMERSON 1918).

Dd, Dwarf plant (EMERSON 1912c, EMERSON and EMERSON 1922).

$L_{g}l_{g}$, Liguleless leaf (EMERSON 1912b).

S_hs_h, Shrunken endosperm (HUTCHISON 1921, 1922 b).

S_us_u, Sugary endosperm (EAST and HAYES 1911).

W_xw_x, Waxy endosperm (COLLINS 1909).

Yy, Yellow endosperm (EAST and HAYES 1911).

Linkage Tests with Pale Green 2

Linkage between $P_{g2}p_{g2}$ and *Dd*

A cross was made between homozygous $p_{g2}p_{g2}$ and a homozygous *dd* plant, the F_2 data being given in table 13. From these data it can be seen that $P_{g2}p_{g2}$ and *Dd* are linked. The difference from expectancy is very great when calculations are made on the assumption that $P_{g2}p_{g2}$ and *Dd* are inherited independently, χ^2 being 102.056. The chances that this difference is due to random sampling are negligible. On the other hand, if calculations are made with the assumption that there is a linkage between $P_{g2}p_{g2}$ and *Dd* with about 32 percent of crossing over, the fit is very close, χ^2 being less than one and P more than .801. The percentage of crossing over is calculated by EMERSON'S method (EMERSON 1916).

Dwarf is a useful character, known for a long time in maize, which has been frequently used in linkage tests, but has not as yet been found to be linked with any character. The linkage relation between *Dd* and $P_{g2}p_{g2}$ with 32 percent of crossing over may be helpful in connecting *Dd* with some other linkage group or in establishing an independent group.

Test between $P_{g2}p_{g2}$ and W_xw_x

Table 14 gives the backcross data involving $P_{g2}p_{g2}$ and W_xw_x, from which it can be seen that there is a close agreement between calculated and observed data when the calculation is made with the assumption that these two factors are inherited independently. The difference from equality between parental and recombination classes is 10.00 ± 6.47, which justifies the conclusion that $P_{g2}p_{g2}$ and W_xw_x are not closely linked.

Tests between $P_{g2}p_{g2}$ and S_hs_h, *Cc*, *Rr*, S_us_u, *Yy*

The summary of F_2 data of linkage tests between $P_{g2}p_{g2}$ and S_hs_h, *Cc*, *Rr*, S_us_u, *Yy*, is given in table 3, from which it can be noted that in all

cases there exists a close agreement between calculated and observed values. The calculations were made on the basis of independent inheritance and that seems to justify the conclusion that $P_{g2}p_{g2}$ is inherited independently from S_hs_h, *Cc*, *Rr*, S_us_u and *Yy*.

TABLE 3

Summary of data from linkage tests between P_{g2} p_{g2} and S_hs_h, Cc, Rr, S_us_u and Yy.

FACTORS INVOLVED		$P_{g2}X$*	$p_{g2}X$	$P_{g2}x$	$p_{g2}x$	TOTAL	χ^2	P	NUMBER OF TABLE WHERE DETAILED DATA ARE GIVEN
S_hs_h	Observed	252	77	64	25	418	3.931	.270	15
	Calculated 9 : 3 : 3 : 1	*235.1*	*78.4*	*78.4*	*26.1*				
Cc and *Rr*	Observed	295	86	205	68	654	2.314	.516	16
	Calculated 27 : 9 : 21 : 7	*275.9*	*92*	*214.6*	*71.5*				
S_us_u	Observed	348	120	96	37	601	3.232	.259	17
	Calculated 9 : 3 : 3 : 1	*338.1*	*112.7*	*112.7*	*37.5*				
Yy	Observed	1496	500	448	149	2593	5.416	.147	18
	Calculated 9 : 3 : 3 : 1	*1458.5*	*486.2*	*486.2*	*162.1*				

* *Xx* stands for any of the factors listed in the first column.

Linkage Tests with Pale Green 3

Linkage between $P_{g3}p_{g3}$ and B_nb_n

Table 19 gives F_2 data from a cross $P_{g3}P_{g3}$ $B_nB_n \times p_{g3}p_{g3}b_nb_n$, which shows conclusively that $P_{g3}p_{g3}$ and B_nb_n are closely linked. The percentage of crossing over as calculated by EMERSON'S method (EMERSON 1916) is 4.53. If the calculation is made with that assumption the fit between calculated and observed data is very good, χ^2 being less than one and P more than .801.

Linkage Tests with Pale Green 4

Tests between $P_{g4}p_{g4}$ and W_xw_x, *Cc*, *Rr*, *Aa*, L_gl_g, *Yy* and S_us_u are summarized in table 4. All calculations were made assuming the in-

dependent inheritance of the involved factors and they closely agree with the observed data. In the case of the cross between $P_{g4}p_{g4}$ and $S_u s_u$, sugary seeds had a poor germination, as frequently happens. This made

TABLE 4

Summary of data from linkage tests between P_g, p_g, and W_x w_x, Cc, Rr, Aa, $L_g l_g$, Yy and $S_u s_u$.

FACTORS INVOLVED		$P_{g4}X$*	$p_{g4}Y$	$P_{g4}x$	$p_{g4}y$	TOTAL	χ^2	P	NUMBER OF TABLE WHERE DETAILED DATA ARE GIVEN
$W_x w_x$	Observed	1300	396	410	131	2237	3.333	.349	20
	Calculated 9 : 3 : 3 : 1	*1259*	*419*	*419*	*140*				
Cc and *Rr*	Observed	556	185	471	149	1361	2.129	.549	21
	Calculated 27 : 9 : 21 : 7	*574*	*191.5*	*446.5*	*149*				
Aa and *Rr*	Observed	579	178	408	124	1289	4.878	.183	22
	Calculated 27 : 9 : 21 : 7	*543.9*	*181.3*	*422.9*	*140.9*				
Rr	Observed	368	106	113	38	625	1.819	.614	23
	Calculated 9 : 3 : 3 : 1	*352*	*117*	*117*	*39*				
$L_g l_g$	Observed	556	171	178	56	960	1.346	.724	24
	Calculated 9 : 3 : 3 : 1	*540*	*180*	*180*	*60*				
Yy	Observed	800	244	271	85	1400	1.847	.607	25
	Calculated 9 : 3 : 3 : 1	*787.5*	*262.5*	*262.5*	*87.5*				
$S_u s_u$	Observed	924	285	198	71	1478			26
	Calculated 3 : 1 ; 3 : 1	*906.75*	*302.25*	*201.75*	*67.25*				
	Difference	17.25 ± 10.1		3.75 ± 4.79					

* *Xx* stands for any of the factors listed in the first column.

the sugary classes lower and it seemed more feasible to consider them separately from non-sugary classes in all calculations. The observed data in all linkage tests agree fairly well with the calculated data, which seems to justify the conclusion that $P_{g^1}p_{g^1}$ is independently inherited from W_xw_x, *Cc*, *Rr*, *Aa* L_gl_g, *Yy* and S_us_u.

DISCUSSION

From a physiological standpoint all pale green types found in maize, as well as in other plants, can be placed in one of the following groups:

1. Deficient types not having enough chlorophyll for photosynthesis.
2. Vital types, having enough chlorophyll for photosynthesis and remaining pale green to maturity.
3. Expallescent types, having enough chlorophyll for photosynthesis, but dying in the early seedling stage.
4. Progressive types, which are pale green in the early seedling stage, the amount of chlorophyll developing with age.
5. Degressive types, in which the amount of chlorophyll diminishes with age.

The distinction between the first and the third group is not very sharp, because it is hard to determine whether the plants died because they did not have enough chlorophyll or because they were expallescent. According to observations made by CORRENS (1919 a) on *Capsella bursa-pastoris* and by TRAJKOVICH (1924) on maize, plants having as little as 10 percent of normal chlorophyll content may live if grown in favorable conditions. It was found in both cases that these plants were very weak, and it is hardly probable that a lower amount of chlorophyll would be adequate for sufficient photosynthesis. Ten percent of normal chlorophyll may be considered, therefore, as the upper limit for the first group and all lethal types having 10 percent or more of normal chlorophyll can be put in the third group.

The pale green types belonging to the second group are numerous. They include, for example, *xantha* in maize (TRAJKOVICH 1924), the pale green types described in Mirabilis, *Capsella bursa-pastoris*, Arabis, Ipomea, Tropaeolum and Urtica by CORRENS (1909, 1919 a, 1919 b, 1920, 1922) and the two complementary pale green types, *chlorina* and *pallida*, described by SHULL (1914) in *Lychnis* (*Melandrium*) *dioica*.

Expallescent, the name given to the third group of pale green types, was first applied by CORRENS (1919 b) to designate the types which are pale green at the beginning of life but soon lose their chlorophyll and die. In

this paper it is used as a name for types which, in spite of having enough chlorophyll for photosynthesis, die in the early seedling stage. The best example of this group is pale green 5 which has about 80 percent of normal chlorophyll and practically normal xanthophyll and carotin content, but still can live no longer than white seedlings which do not possess any chlorophyll at all. The size which these seedlings reach before they die is apparently entirely dependent on the size of the seeds, that is, on the amount of reserve food. The cause of the death of these seedlings is not known, but it is probable that in them the chlorophyll does not develop to the stage in which it can function in photosynthesis.

From seven pale green types described in maize, four ($P_{g1}p_{g1}$, $P_{g3}p_{g3}$, $P_{g4}p_{g4}$ and $P_{g5}p_{g5}$) belong to the expallescent group. This may be due to the fact that expallescent types were picked out by chance in selecting material for this study, or it may well be that they occur more frequently than the other types. It is easy to conceive that a mutation which affects the chlorophyll would most likely hinder chlorophyll development rather than stimulate it, and it seems probable that mutations having stronger detrimental effects would be more frequent than those which were less injurious. If this is the case, then it may be expected that white seedlings, as the most detrimental type of chlorophyll mutations, would be most frequent, more so than pale green, and still more than variegated and virescent types. Among pale green seedlings we should expect the expallescent types to be the most frequent ones. Maize affords excellent material to test these points, as cross-pollination, which is a more or less facultative method of fertilization in this species, tends to preserve weak mutations in the heterozygous condition and has allowed them to accumulate in this species during the centuries of its cultivation. The recessive types can be brought to light again upon selfing, and if this is done on a large scale by random sampling of as many different varieties as possible, a fair picture of the frequency of different types can be obtained. This was done by HUTCHISON (1922 a) who found that from 1872 selfed ears, representing 468 varieties, 144 gave progenies that segregated white seedlings, 107 pale greens, 58 different variegated, 57 virescent and 164 miscellaneous chlorophyll types. As was expected, white seedlings were found to be the the most frequent of all the chlorophyll types, which lends indirect support to the above hypothesis accounting for the prevalence of the expallescent class among the pale green types.

The progressive group of the pale green types, of which pale green 2 is an example, has a parallel in the virescent group (LINDSTROM 1918, DEMEREC 1924), the main difference being that in the first one the primary

color is pale green and in the second white or yellowish. In both cases the mature plants are green and can not be distinguished from the other green plants.

Golden in maize is a good representative of the degressive group of pale green types. Golden plants are normally green when young, becoming more and more yellow with age. The disappearance of chlorophyll is slow and progressive, as is seen in the case of a plant having the older leaves yellower than the younger ones. When a chlorophyll test was made on a plant in the tasselling stage it was found that the upper leaves possessed about 50 percent and the lower ones about 9 percent of normal chlorophyll, the content of xanthophyll and carotin being normal. A similar degressive condition in chlorophyll development was found in zebra-striped maize (DEMEREC 1921, STROMAN 1924); in that case, however, the disappearance of chlorophyll is only partial, leaving transverse zebra-like stripes throughout the leaves.

SUMMARY

Five pale green types of maize were analyzed genetically, all possible inter-crosses were made, to show that they are different.

The xanthophyll and carotin content in all investigated types were found to be practically normal, the difference between the various types being in the different chlorophyll content.

Pale green 2 ($P_{g2}p_{g2}$) has about 50 percent of normal chlorophyll. It was found to be linked with dwarf (*Dd*) (crossing over about 32 percent) and inherited independently of S_hS_h, *Cc*, *Rr*, S_uS_u and *Yy*.

Pale green 3 ($P_{g3}p_{g3}$) has about 30 percent of normal chlorophyll content. It is linked with brown aleurone color (B_nb_n) with 4.5 percent of crossing over.

Pale green 4 ($P_{g4}p_{g4}$) has about 50 percent of normal chlorophyll content. Linkage tests show that it is inherited independently of W_zw_z, *Cc*, *Rr*, *Aa*, L_gl_g, *Yy* and S_uS_u.

Pale green 5 ($P_{g5}p_{g5}$) has about 80 percent of normal chlorophyll content.

Xantha 2 ($X_{n2}x_{n2}$) has about 10 percent of normal chlorophyll and resembles *xantha* already described in maize.

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APPENDIX—TABLES 5 to 26

TABLE 5

F_2 data from a cross $p_{g1}p_{g2}$ $P_{g1}P_{g1} \times P_{g1}P_{g1}$ $P_{g1}p_{g2}$.

PEDIGREE NUMBER	P_g	p_g	TOTAL	CALCULATED p_g	DIFFERENCE
9 : 7 ratios					
1037– 1	171	122	293	..	..
– 2	136	101	237	..	..
– 3	83	61	144	..	..
– 7	85	63	148	..	..
– 9	168	116	284	..	..
–10	107	75	182	..	..
–11	156	137	293	..	..
–16	131	103	234	..	..
–18	197	115	312	..	..
Total	1234	893	2127	931	34.00±15.45
3 : 1 ratios					
1037– 4	154	48	202	..	..
– 5	199	62	261	..	..
– 6	151	49	200	..	..
– 8	154	52	206	..	..
–12	207	70	277	..	..
–13	107	36	143	..	..
–14	58	18	76	..	..
–15	131	48	179	..	..
–17	84	26	110	..	..
–19	82	28	100	..	..
Total	1327	437	1754	438	1.0±12.2

TABLE 6

F_2 data from a cross $p_{g_1}p_{g_2}P_{g_3}P_{g_4}\times P_{g_1}P_{g_2}P_{g_3}p_{g_4}$.

PEDIGREE NUMBER	P_g	p_g	TOTAL	CALCULATED p_g	DIFFERENCE
9 : 7 ratios					
1044– 3	163	135	298	..	..
– 5	241	200	441	..	..
– 6	236	183	419	..	..
– 7	90	83	173	..	..
– 8	186	129	315	..	..
–12	208	166	374	..	..
–14	87	93	180	..	..
–15	132	117	249	..	..
–18	118	94	212	..	..
–22	106	81	187	..	..
Total	1567	1281	2848	1246	35.00±17.85
3 : 1 ratios					
1044– 2	177	60	237	..	..
– 4	184	58	242	..	..
– 9	70	18	88	..	..
–11	126	51	177	..	..
–19	129	44	173	..	..
Total	686	231	917	229.25	1.75±8.84

TABLE 7

F_2 data from a cross $P_{g_1}P_{g_2}P_{g_3}p_{g_4}\times P_{g_1}p_{g_2}P_{g_3}P_{g_4}$.

PEDIGREE NUMBER	P_g	p_g	TOTAL	CALCULATED p_g	DIFFERENCE
9 : 7 ratios					
1039– 1	168	129	297	..	..
– 3	185	139	324	..	..
Total	353	268	621	271.7	3.70±8.34
3 : 1 ratios					
1039– 2	78	23	101	..	..
– 4	54	19	73	..	..
– 8	94	25	119	..	..
–12	89	28	117	..	..
–14	103	34	137	..	..
–15	39	16	55	..	..
–17	49	16	65	..	..
Total	506	161	667	166.75	5.75±7.54
Homozygous for green					
1039–11					
–13					

TABLE 8

F_2 data from a cross $p_{g_1}p_{g_2}$ $X_{n_1}X_{n_2}\times P_{g_1}P_{g_2}$ $X_{n_1}v_{n_2}$.

PEDIGREE NUMBER	P_g	p_g	TOTAL	CALCULATED p_g	DIFFERENCE
9 : 7 ratios					
1603–1	144	107	251	..	..
–2	109	108	217	..	..
–4	37	24	61	..	..
–5	58	40	98	..	..
Total	348	279	627	274.3	4.70±8.38
3 : 1 ratio					
1603–3	117	33	150	37.5	4.50±3.58

TABLE 9

F_2 data from a cross $P_{g_1}P_{g_2}$ $P_{g_1}p_{g_2}\times P_{g_1}p_{g_2}$ $P_{g_1}P_{g_2}$.

PEDIGREE NUMBER	P_g	p_g	TOTAL	CALCULATED p_g	DIFFERENCE
9 : 7 ratios					
1042– 2	165	152	317	..	..
–12	206	153	359	..	..
Total	371	305	676	295.75	9.25±8.70
3 : 1 ratios					
1042– 4	109	46	155	..	..
– 6	129	41	170	..	..
– 8	47	15	62	..	..
– 9	418	135	553	..	..
–14	230	80	310	..	..
Total	933	317	1250	312.5	4.5±10.3
Homozygous for green					
1042– 1					
– 3					
– 5					
– 7					
–11					
–13					

TABLE 10

F_2 data from a cross $P_{g_1}p_{g_2}$ $P_{g_3}P_{g_4}$×$P_{g_3}P_{g_4}$ $P_{g_5}p_{g_6}$.

PEDIGREE NUMBER	P_g	p_g	TOTAL	CALCULATED p_g	DIFFERENCE
9 : 7 ratio					
1605–1	73	55	128	56	1.00±3.79
3 : 1 ratio					
1605–2	112	33	145	36.25	3.25±3.52

TABLE 11

F_2 data from a cross $P_{g_1}p_{g_2}$ $P_{g_3}P_{g_4}$×$P_{g_3}P_{g_4}$ $P_{g_5}p_{g_6}$.

PEDIGREE NUMBER	P_g	p_g	TOTAL	CALCULATED p_g	DIFFERENCE
9 : 7 ratios					
1041– 6	237	176	413	..	..
–17	237	177	414	..	..
–21	214	162	376	..	..
–10	37	26	63	..	..
Total	725	541	1266	554	13.00±11.85
3 : 1 ratios					
1041– 3	47	20	67	..	..
– 5	50	17	67	..	..
– 7	48	18	66	..	..
– 9	204	73	277	..	..
–12	51	14	65	..	..
–15	48	12	60	..	..
–18	40	13	53	..	..
–22	41	18	59	..	..
Total	529	185	714	178.5	6.5±7.8
Homozygous for green					
1041– 2					
– 4					
– 8					
–13					
–14					
–16					
–19					
–20					

TABLE 12

F_2 data from a cross $X_{n_1}x_{n_1}\ X_{n_2}X_{n_2} \times X_{n_1}X_{n_1}\ X_{n_2}x_{n_2}$.

PEDIGREE NUMBERS	X_n	x_n	TOTAL	CALCULATED x_n	DIFFERENCE
9 : 7 ratios					
1611–1	103	84	187	..	..
–3	101	82	183	..	..
–6	118	93	211	..	..
Total	322	259	581	254.2	4.80±8.07
3 : 1 ratios					
1611–2	73	28	101	..	..
–5	73	23	96	..	..
Total	146	51	197	49.25	1.75±4.10
Homozygous for green					
1611–4					
–7					

TABLE 13

F_2 data from a cross $p_{g_1}p_{g_1}\ Dd \times P_{g_1}P_{g_1}\ dd$, showing linkage between $P_{g_1}p_{g_1}$ and Dd with about 32 percent of crossing over.

PEDIGREE NUMBERS	D		d		TOTAL
	P_{g_1}	p_{g_1}	P_{g_1}	p_{g_1}	
1013– 2	171	74	73	8	326
– 4	176	78	66	4	324
– 7	246	107	97	9	459
–12	247	99	107	11	464
–16	269	118	122	19	528
–19	255	108	115	14	492
Total	1364	584	580	65	2593
Calculated, 9 : 3 : 3 : 1	*1458.5*	*486.2*	*486.2*	*162.1*	
Difference	−94.5	+97.8	+93.8	−97.1	
	χ^2=102.056				
Calculated with 32 percent of crossing over	*1363.01*	*581.74*	*581.74*	*66.59*	
Difference	+.99	+2.22	−1.74	−1.51	
	χ^2<1	P>.801			

TABLE 14

Backcross data from a cross $P_{g_1}p_{g_1}W_xw_x \times p_{g_1}p_{g_1}w_xw_x$, showing that $P_{g_1}p_{g_1}$ and W_xw_x are inherited independently.

PEDIGREE NUMBER	PARENTAL COMBINATIONS		RECOMBINATIONS		TOTAL
	$W_x\ p_{g_1}$	$w_x\ P_{g_1}$	$W_x\ P_{g_1}$	$w_x\ p_{g_1}$	
1016– 5×1020–2	30	33	36	40	139
–12× –2	49	62	68	50	229
Subtotal	79	95	104	90	368
Total	174		194		
Calculated, 1 : 1	*184*		*184*		
Deviation	10.00±6.47				

TABLE 15

F_2 data from a cross $P_{g_1}P_{g_1}\ s_hs_h \times p_{g_1}p_{g_1}\ S_hS_h$, showing that $P_{g_1}p_{g_1}$ and S_hs_h are inherited independently.

PEDIGREE NUMBER	S_h		s_h		TOTAL
	P_{g_1}	p_{g_1}	P_{g_1}	p_{g_1}	
1023– 1	88	29	14	4	135
– 4	85	24	22	12	143
–11	79	24	28	9	140
Total	252	77	64	25	418
Calculated, 9 : 3 : 3 : 1	*235.1*	*78.4*	*78.4*	*26.1*	
Difference	+16.9	−1.4	−14.4	−1.1	

$\chi^2 = 3.9311$ P=0.2704

TABLE 16

F_2 data from a cross $P_{g_1}P_{g_1}$ CC RR×$p_{g_1}p_{g_1}$ cc rr.

PEDIGREE NUMBER	COLORED SEEDS		COLORLESS SEEDS		TOTAL
	P_{g_1}	p_{g_1}	P_{g_1}	p_{g_1}	
1028–1	137	33	96	32	298
–2	158	53	109	36	356
Total	295	86	205	68	654
Calculated, 27 : 9 : 21 : 7	*275.9*	*92*	*214.6*	*71.5*	
Difference	+19.1	−6.0	−9.6	−3.5	

$\chi^2 = 2.3143$ P=0.5156

TABLE 17

F_2 data from a cross $P_{g_1}P_{g_2}\ s_u\ s_u \times p_{g_1}p_{g_2}\ S_u\ S_u$, showing that $P_{g_1}p_{g_2}$ and $S_u\ s_u$ are inherited independently.

PEDIGREE NUMBER	S_u		s_u		TOTAL
	P_{g_2}	p_{g_2}	P_{g_2}	p_{g_2}	
1016–1	152	60	50	19	281
–4	196	60	46	18	320
Total	348	120	96	37	601
Calculated, 9 : 3 : 3 : 1	*338.1*	*112.7*	*112.7*	*37.5*	
Difference	+9.9	+7.3	−16.7	−.5	

$\chi^2 = 3.232$ P = 0.259

TABLE 18

F_2 data from a cross $p_{g_1}p_{g_2}\ Yy \times P_{g_1}P_{g_2}\ yy$, showing that $P_{g_1}p_{g_2}$ and Yy are inherited independently.

PEDIGREE NUMBER	Y		y		TOTAL
	P_{g_2}	p_{g_2}	P_{g_2}	p_{g_2}	
1013– 2	186	65	58	17	326
– 4	185	60	57	22	324
– 7	268	89	75	27	459
–12	257	79	97	31	464
–16	315	111	76	26	528
–19	285	96	85	26	492
Total	1496	500	448	149	2593
Calculated, 9 : 3 : 3 : 1	*1458.5*	*486.2*	*486.2*	*162.1*	
Difference	+37.5	+13.8	−38.2	−13.1	

$\chi^2 = 5.4158$ P = 0.1468

TABLE 19

F_2 data from a cross $P_{g_1}P_{g_2}\ B_n\ B_n \times p_{g_1}p_{g_2}\ b_n\ b_n$, showing linkage between $P_{g_1}p_{g_2}$ and $B_n\ b_n$, with about 4.53 percent of crossing over.

PEDIGREE NUMBER	B_n		b_n		TOTAL
	P_{g_2}	p_{g_2}	P_{g_2}	p_{g_2}	
1042–9	203	8	5	65	281
Calculated, 4.53 percent of crossing over	*204.8*	*6.1*	*6.1*	*64.0*	
Difference	−1.8	+1.9	−1.1	+1.0	

$\chi^2 < 1.0$ P > 0.8013

TABLE 20

F_2 data from a cross $P_{g_4}P_{g_4}$ w_z $w_z \times p_{g_4}$ p_{g_4} W_z W_z, showing that $P_{g_4}p_{g_4}$ and W_z w_z are inherited independently.

PEDIGREE NUMBER	W_z		w_z		TOTAL
	P_{g_4}	p_{g_4}	P_{g_4}	p_{g_4}	
1030– 6	123	46	40	18	227
– 7	213	62	62	20	357
– 8	150	45	41	14	250
–19	155	47	54	16	272
–24	179	46	54	15	294
1033– 6	124	48	53	10	235
–10	99	28	31	11	169
–11	127	34	34	10	205
–12	130	40	41	17	228
Total	1300	396	410	131	2237
Calculated, 9 : 3 : 3 : 1	*1259*	*419*	*419*	*140*	
Difference	+41	−23	−9	−9	

$\chi^2 = 3.333$ $P = 0.349$

TABLE 21

F_2 data from a cross $P_{g_4}P_{g_4}$ RR cc $\times p_{g_4}p_{g_4}$ rr CC showing that $P_{g_4}p_{g_4}$ is inherited independently of Rr and Cc.

PEDIGREE NUMBER	COLORED SEEDS *CR*		COLORLESS SEEDS *cR*, *Cr* and *cr*		TOTAL
	P_{g_4}	p_{g_4}	P_{g_4}	p_{g_4}	
1031– 1	57	13	35	16	121
–16	98	30	95	28	251
– 5	108	32	87	26	253
– 9	98	35	74	25	232
–15	76	27	70	19	192
– 3	119	48	110	35	312
Total	556	185	471	149	1361
Expected, 27 : 9 : 21 : 7	*574*	*191.5*	*446.5*	*149*	
Difference	−18	−6.5	+24.5	0	

$\chi^2 = 2.129$ $P = 0.549$

TABLE 22

F_2 data from a cross $P_{a_1}P_{a_4}$ aa RR×$p_{a_2}p_{a_3}$ rr AA, showing that $P_{a_1}p_{a_4}$ is inherited independently from Aa and Rr.

PEDIGREE NUMBER	COLORED SEEDS *AR*		COLORLESS SEEDS *aR*, *AR* and *ar*		TOTAL
	P_a	p_a	P_a	p_a	
1029– 3	175	57	135	39	406
– 4	145	44	91	32	312
– 6	110	31	75	23	239
–10	149	46	107	30	332
Total	579	178	408	124	1289
Calculated, 27 : 9 : 21 : 7	*543.9*	*181.3*	*422.9*	*140.9*	
Difference	+35.1	−3.3	−14.9	−16.9	

χ^2=4.878 P=0.183

TABLE 23

F_2 data from a cross $P_{a_1}P_{a_4}$ RR×$p_{a_2}p_{a_3}$ rr, showing that $P_{a_1}p_{a_4}$Rr are inherited independently.

PEDIGREE NUMBER	*R*		*r*		TOTAL
	P_{a_1}	p_{a_4}	P_{a_1}	p_{a_4}	
1034– 5	175	59	48	19	301
–24	193	47	65	19	324
Total	368	106	113	38	625
Calculated, 9 : 3 : 3 : 1	*352*	*117*	*117*	*39*	
Difference	+16	−11	−4	−1	

χ^2=1.819 P=0.614

TABLE 24

F_2 data from a cross $P_{g^4}P_{g^4}\, l_g l_g \times p_{g^4}p_{g^4}\, L_g L_g$, showing that $P_{g^4}p_{g^4}$ and $L_g l_g$ are inherited independently.

PEDIGREE NUMBER	L_g		l_g		TOTAL
	P_{g^4}	p_{g^4}	P_{g^4}	p_{g^4}	
1034– 1	146	41	42	16	245
– 5	165	59	58	19	301
–15	47	19	18	6	90
–24	198	52	60	14	324
Total	556	171	178	55	960
Calculated, 9 : 3 : 3 : 1	*540*	*180*	*180*	*60*	
Difference	+16	−9	−2	−5	

$\chi^2 = 1.346$ $\quad P = 0.724$

TABLE 25

F_2 data from a cross $P_{g^4}P_{g^4}\, yy \times p_{g^4}p_{g^4}\, YY$, showing that $P_{g^4}p_{g^4}$ and Yy are inherited independently.

PEDIGREE NUMBER	Y		y		TOTAL
	P_{g^4}	p_{g^4}	P_{g^4}	p_{g^4}	
1030– 6	120	53	43	11	227
– 7	208	55	67	27	357
– 8	142	46	49	13	250
–19	153	48	56	15	272
–24	177	42	56	19	294
Total	800	244	271	85	1400
Calculated, 9 : 3 : 3 : 1	*787.5*	*262.5*	*262.5*	*87.5*	
Difference	+12.5	−18.5	+8.5	−2.5	

$\chi^2 = 1.847$ $\quad P = 0.607$

TABLE 26

F_2 data from a cross $P_{g4}P_{g4}\ s_u\ s_u \times P_{g4}p_{g4}\ S_u\ S_u$, showing that $P_{g4}p_{g4}$ and $S_u\ s_u$ are inherited independently.

PEDIGREE NUMBER	S_u		s_u		TOTAL
	P_{g4}	p_{g4}	P_{g4}	p_{g4}	
1033– 6	177	58	43	13	291
–10	130	39	27	8	204
–11	161	44	36	17	258
–12	171	57	18	6	252
1031– 1	92	29	19	9	149
–16	133	58	55	18	324
Total	924	285	198	71	1478
Calculated, 3 : 1	*906.75*	*302.25*	*201.75*	*67.25*	
Difference	17.25±10.10		3.75±4.79		

PROPHASE CHROMOSOME BEHAVIOR IN TRIPLOID INDIVIDUALS OF *DROSOPHILA MELANOGASTER*

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One feature of chromosome behavior characteristic of the Diptera is the paired association of homologous chromosomes in the somatic cells as well as in the germ cells. This is indicated by the side-by-side arrangement of homologues in the metaphase plate (STEVENS 1907, 1908), which is in turn due to an intimate association in the prophase when the chromosomes are drawn out into long threads (METZ 1916). It has been found that this same principle of association of homologues applies in the case of tetraploid cells (somatic) in flies (HOLT 1917, METZ 1916, 1922). Here the association is in fours, and apparently all four members of a set are associated with equal intimacy (METZ 1922). In octaploid cells it is probable that a similar association occurs, the sets being composed of eight members (METZ 1916, HOLT 1917), but the evidence is not so clear here since only metaphases were identified as octaploid.

These results seem to indicate that the association is due to the attraction of homologous materials in corresponding chromosomes, and not to a simple positive-negative type of attraction which would become "balanced" when two members came together. In all of the above cases, however, homologous chromosomes have been present in even numbers. No flies have hitherto been available in which odd numbers were present. Such flies are now provided, however, by the triploid "race" of *Drosophila melanogaster* obtained by Doctor C. B. BRIDGES, who has kindly furnished material for the present study.

An examination of this material has shown that here also homologous chromosomes are not only associated in metaphase (cf. figure 4, and also figures in a forthcoming paper by BRIDGES), but undergo an intimate association during somatic prophases, the association being in threes, and all three members being associated with equal intimacy, as far as can be determined visually. The inferences drawn from previous observations thus apply to this case in which homologues are present in odd numbers.

Explanation of Plate 1

All figures are from sectioned material, fixed in strong Flemming. They were drawn with the aid of the camera lucida, at table level, using Zeiss 1.5 mm objective and number 12 ocular, and are reproduced without reduction.

All figures are from *Drosophila melanogaster* Meigen.

Figures 1 to 3 are from diploid specimens; the remainder are from triploids.

Figures 1 to 3 represent the metaphase and prophase conditions in ordinary diploid specimens.

Figure 4 is a typical triploid metaphase with an extra Y chromosome ($3n+Y$). The larger of the small bodies in the center is probably double, representing two of the three "m chromosomes." All of the other chromosomes are obviously in threes, except the extra Y at the top of the figure.

Figures 5, 6, 7, 10 and 11 are prophases from adjacent cells near the tip (distal end) of an ovary (slide 2165-2). These are undifferentiated cells. Figure 5, and probably figures 6 and 7, represent entire or practically entire nuclei, although in 7 part of one or two chromosomes may be cut off. In each of these figures the triads are slightly transposed to prevent overlapping. No good metaphases were obtained in this ovary, but the prophases have an extra small body which presumably is a Y chromosome. This body appears to be close to the X's in the drawings (figures 5, 6, 7), but the appearance is somewhat misleading, for in figure 5 the X's are transposed and should be farther from the Y-like body, and in 7 the body is at a higher level than the X's. In figure 6 the relative positions are correctly represented.

Figures 8 and 9 are from ovarian follicle cells. Here again the Y chromosome may be present, although no good metaphases were obtained to check the point. In figure 9 only the large autosomes are clear. The sex chromosomes are either cut or broken apart or otherwise disturbed, so that their relations are not clear.

Figures 10 and 11 represent single triads from triploid prophases to show the intimacy of association of the three homologues.

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A detailed discussion of these earlier cases in the flies, including theoretical considerations and also a comparison with analogous conditions in other organisms, has been given in earlier papers (METZ 1916, 1922). The present paper will, therefore, be mainly a descriptive account.

The nature of the prophase association in triploid cells is best shown by the figures, and needs little discussion. In the following account a résumé of the general features is given first, followed by notes on the individual figures.

Typical prophase figures are shown in the accompanying camera lucida drawings, which were made from somatic and ovarian cells of females,[1] including follicle cells and undifferentiated cells whose descendants become nurse cells and oöcytes. Chromosome behavior is not visibly different in these different types of cells.

Figures 1 to 3 are typical diploid figures, the latter two showing the close approximation of homologues in prophase. The remainder are triploid figures. These are from specimens identified as triploids by their external appearance and (in some cases) by metaphase chromosome groups, as well as by the prophase figures shown here; so it is safe to assume the presence of the triploid number even when the association is so close that a direct count cannot be made.

In some, if not all, of the accompanying triploid figures an extra Y chromosome is present. This is shown clearly in the metaphase group of figure 4. Such groups, therefore, are $3n+Y$. Apparently the Y does not associate with the three X's in prophase, although it may be near them (see below). The very small "m" or "fourth" chromosomes are not, as a rule, distinguishable in the prophases, either in triploid or diploid cells.

Most of the accompanying prophase figures represent a fairly late stage in prophase, and in most cases some of the triads are transposed in drawing so that they will not overlap. In the actual figures they usually lie at different levels and overlap. It was found impracticable to draw earlier stages because the threads were so elongate and intertwined that they could not be represented clearly. From analogy with conditions in diploid cells of other Diptera (METZ 1916), however, it is assumed that the association of homologues is more intimate in the earlier prophase stages than in those figured here.

The autosomes appear to associate uniformly throughout their length, but the X chromosomes seem to behave differently. In most figures the latter are closely associated for about half their length, and then diverge,

[1] All of the triploids are females. See forthcoming paper of BRIDGES.

finger-like, as shown by the drawings. This may be due to a precocious separation of the homologues at one end, preceded by a stage of close association throughout. An attempt is being made to study this feature further. The figures thus far examined indicate that the behavior is constant and that the end at which the association remains intimate is probably the one at which the spindle fibres become attached. In any case, the phenomenon is sufficiently definite to suggest that it is a reflection of the process in oögenesis which is responsible for the unusual crossover values given by the X chromosome in triploid flies, as is pointed out by BRIDGES in a later paper. In such flies BRIDGES has found that crossing over near one end of the X is greater, and near the other end less, than in ordinary diploid flies.

In late prophase, as the chromosomes separate and go on the spindle, the X's (which are rod-like and have a terminal spindle-fibre attachment) tend to retain their positions with respect to one another, usually remaining parallel. The large autosomes, however, are apparently disturbed in their arrangement. These are V-shaped, and have a median spindle-fibre attachment. Instead of remaining parallel and going on the spindle "three deep," as might be expected, they frequently, or usually, separate. Judging from the available figures one of the most frequent shifts results in the condition shown in figure 4, in which one member of each set has moved to one side,—the other two remaining parallel as in diploid figures.

This behavior suggests that the equator of the spindle is restricted in size, and that the autosomes cannot remain "three deep" without either extending beyond the margin of the equator or else crowding too closely together in the center. In other words, it suggests that the chromosomes move, or are drawn, to within a definite distance of the center, but are at the same time held apart so that they are not crowded. Apparently, an equilibrium cannot, as a rule, be reached if the autosomes all remain parallel and three deep.

If, as suggested by observations on other Diptera (METZ and NONIDEZ 1922, 1924), each chromosome is enveloped in a transparent gelatinous sheath or rind, these sheaths may hold them apart and may account for the apparent absence of crowding near the center of the figures,—which in turn may account for the shifting of some chromosomes out of alignment.

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ALCOHOL AND THE SEX RATIO

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	351
Experimental results	352
Discussion of the literature	355
CONCLUSION	357
LITERATURE CITED	357

INTRODUCTION

This paper deals with the effect of alcohol upon the sex ratio in albino rats. It is one of a series of papers on the effects of alcohol upon the sex ratio, litter size, birth weight, growth curve and duration of life in white rats.

The data cover ten successive generations of control and alcoholic animals. Comparisons are made, generation by generation, and the sex ratios for the total number of control and treated are given and discussed. This experiment was started with a single pair of semi-inbred rats obtained from the WISTAR INSTITUTE colony. All the 3123 animals included in the sex-ratio data were derived from this single pair of rats. Sister-by-brother mating within the litter was employed throughout the entire experiment.

Alcohol was administered by the now well known fume-tank method. Treatment was severe, i.e., treating an entire litter in a tank, the litter was not removed until most, if not all, of the animals were unable to stand upon their feet. In fact, more often than not the majority of a litter were in a drunken stupor when removed from the treatment tanks. It frequently took them several hours to sleep off the effects. Treatment was commenced at twenty days of age and continued daily until the rats were one hundred days of age. Here treatment stopped and the members of each litter were mated together to produce the next generation. In this way the complications incident to treatment *in utero* were avoided. This work is purely an attack upon the germ cells themselves.

The sex ratios were taken at birth. Litters were looked for usually twice a day, always once a day, and the sex ratios of all new litters re-

corded. These were checked later at twenty days to detect any error in recording. There is, however, little or no error in determining the sex of the new-born rat (JACKSON 1912), so that our sex ratios are approximately one hundred percent accurate. The sex of all still-born rats was, of course, included with the data of the litters of which they were members. A preliminary paper describing further details and the general setting of this experiment was published by HANSON and HANDY in 1923.

EXPERIMENTAL RESULTS

The sex-ratio data given in table 1, and shown graphically in figure 1, are based upon a total of 3123 rats. This is displayed generation by generation and also for the total number of individuals in the control and treated

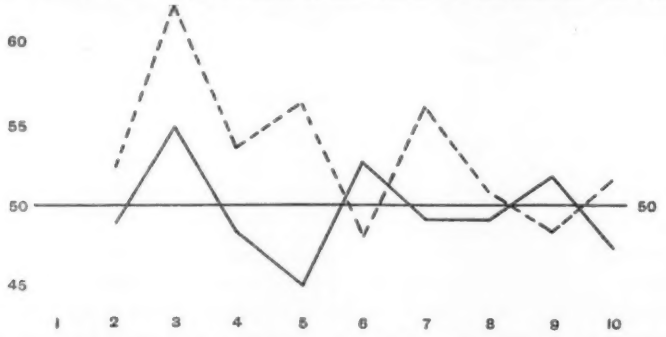

FIGURE 1.—Variability in sex ratio during ten generations of alcoholic treatment. The solid line represents the controls; the broken line, the treated. The abscissal axis represents generations one to ten; the ordinal axis, the sex-ratio percentages.

lines. The sex ratio of the first generation is based upon a single litter and is without significance, being included merely for the sake of completeness of record. In the following generations, however, the numbers are adequate to give reliable sex-ratio data, ranging as they do from one hundred to over three hundred rats per generation.

The formula used for computing the sex ratio is that used by PEARL (1917),

$$\frac{100\,♂♂}{♂♂ + ♀♀},$$

and the probable-error formula from the same source is

$$0.6745\sqrt{\frac{P.Q}{N}}$$

The last column in table 1 gives the difference divided by the probable error, and shows conclusively that in not one of the ten generations is the difference in sex ratio between treated and control of statistical significance. In nine of the ten generations the relation of the difference to its probable error is wholly negligible.

TABLE 1

A table showing sex ratios of control and treated rats of ten successive generations.

GENERATION	CONTROL		TREATED		DIFFERENCE	DIFF. / P.E.
	♂♂ : ♀♀	Percent males	♂♂ : ♀♀	Percent males	+, tests greater −, tests less	
1	2 : 3	40.00±14.78	2 : 3	40.00±14.78	..	..
2	47 : 48	49.30± 3.47	14 : 12	53.80± 6.59	+ 4.50±7.45	..
3	48 : 37	56.47± 3.63	69 : 40	63.30± 4.61	+ 6.83±5.87	1.16
4	89 : 96	48.11± 3.67	120 : 102	54.05± 3.34	+ 5.94±4.96	1.20
5	79 : 100	44.13± 3.71	157 : 117	57.30± 2.99	+13.17±4.76	2.77
6	93 : 82	53.18± 3.77	93 : 99	48.44± 2.43	− 4.74±4.49	1.06
7	103 : 104	49.76± 2.34	52 : 39	57.14± 5.24	+ 7.38±5.74	1.29
8	107 : 108	49.77± 2.30	126 : 120	51.22± 2.15	+ 1.45±3.25	..
9	60 : 55	52.17± 3.15	106 : 113	48.40± 2.28	− 3.77±3.89	..
10	82 : 92	47.13± 2.55	158 : 146	52.13± 1.93	+ 5.00±3.20	1.56
Totals	710 : 725	49.41± 0.89	897 : 791	53.13± 0.82	+ 3.72±1.21	3.07

The fifth generation with a difference of 2.77 times its probable error is the only one even approaching significance, and in the light of the data as a whole even this cannot be considered as having any meaning. Taken generation by generation the evidence is overwhelmingly against any modification of the sex ratio by the treatment employed. In some generations the sex ratios of the treated are higher than the controls, and in other generations this is reversed. The distribution of the high and low sex ratios throughout the entire ten generations seems to be a purely random one.

This situation is somewhat different when the totals for the ten generations are computed. Here the number of animals is very large, with sex ratios of correspondingly low probable errors. The difference between the two sex ratios in the totals (3.72 ± 1.21) is actually small, but is 3.07 times its probable error. Since a difference having a value three times its probable error may be the beginning of a significant difference, this value of 3.07 is somewhat difficult of interpretation.

Inspection of the sex ratios generation by generation does not show any tendency to a higher ratio for the later generations, as might be ex-

pected if the alcohol was adversely affecting the female-producing sperm. On the contrary, the highest sex ratio (63.30 ± 4.61), on the alcoholic side comes in an early generation, the third, while the two generations in which the treated fall below the controls are relatively late, the sixth and ninth. The sex ratio of the tenth alcoholic generation is actually lower than that of the second generation.

Since the high sex ratios of the treated entering into the totals, which seem to give a possibly significant difference to them, lie in the early generations rather than in the later ones, any shift of the sex ratio by means of alcohol must be said to have taken place early in the experiment, at most during the first five generations. But, if so, this was lost during the last five generations. If the modification was an actual one it is difficult to see how this loss could have taken place, as generations 6 to 10 were treated daily and should have retained the gain or even increased it. This they did not do.

In the light of this analysis and also taking into consideration the fact that a difference of three times the probable error is at best only on the threshold of significance, we do not just now see that a very strong case can be made for alcohol as a modifier of the sex ratio.

It is worthy of notice that in the graph both the control and treated lines consistently approach the 50—50 base line in the later generations. This is probably due to the practice of intense inbreeding, only sister-by-brother matings being made throughout the experiment, together with a slight amount of selection of those litters having a nearly equal number of sexes for breeding. This selection factor, however, is very slight, and the decrease in the sex-ratio variability undoubtedly is largely due to the increasing homozygosity of the stock. This is evidenced by the fact that *usually* the first twenty-five or thirty litters in any generation reaching maturity were mated to produce the next generation.

This decrease in variability of the sex ratio in inbred lines is in agreement with the results of KING (1918), who found that in her inbred albino strain A, the variation in sex ratio was much less after the twelfth generation than before. These facts raise the question as to whether an alcoholic effect on the sex ratio might not be overcome or at least neutralized by the opposite tendency to decreased variability introduced by the inbreeding. Apparently, this cannot be answered categorically at this time. It should be pointed out, however, that since the inbreeding factor operated equally in treated and controls there is still left the alcoholic factor as a differential, and this, as we have seen above, apparently had no effect on the sex ratio.

DISCUSSION OF THE LITERATURE

PEARL (1916, 1917, 1924) and STOCKARD and PAPANICOLAOU (1918) are in disagreement as to the effects of alcohol on the sex ratio, PEARL holding that in fowls there was no effect whatever, while STOCKARD maintained that in the guinea-pig alcohol changes the sex ratio. The latter author accounts for the sex-ratio differences by assuming that the poison has a greater affinity for, or is more effective in its action on, one type of sperm than on the other. STOCKARD's conclusion as to the effect of alcohol on the sex ratio is strongly stated as follows:

"The only plausible way to account for the origin of this difference [in the sex ratio] is to assume that the female-producing spermatozoa were more modified by the treatment than the male-producing spermatozoa. Whether such an increased modification is due to the presence of the greater mass of chromatin to be injured in the one case than in the other, or the difference in response on the part of the two heteromorphic sex chromosomes it is impossible to state. *The difference, however, is a fact.*" (Italics ours.)

Since our ten generations of alcoholized rats show no indications whatever of any sex-ratio effect, these results seemed strongly to support PEARL's conclusions with fowls, and to be contrary to what STOCKARD and PAPANICOLAOU reported in guinea-pigs. However, since the latter authors do not reduce any of their data to biometrical constants it occurred to us

TABLE 2

A table showing STOCKARD'S *sex-ratio data reduced to biometrical constants and compared group by group. The raw data are taken from table VI,* STOCKARD *and* PAPANICOLAOU *1918.*

CONTROL	TREATED	DIFFERENCE +, TESTS GREATER −, TESTS LESS
Normal×normal	Normal ♀♀ × treated ♂♂	−2.67±3.84
Normal×normal	Treated ♀♀ × normal ♂♂	−3.91±3.10
Normal×normal	Treated ♀♀ × treated ♂♂	+1.69±3.32
Normal×normal	♀♀ ancestors normal × ♂♂ ancestors treated	−0.90±3.25
Normal×normal	♀♀ ancestors treated × ♂♂ ancestors normal	−6.71±3.44
Normal×normal	♀♀ ancestors treated × ♂♂ ancestors treated	+1.90±3.36
Normal×normal	All classes ♀♀ × all classes ♂♂	−1.74±2.46

to do this in order to see just how great the effect of alcohol upon the sex ratios had been. Taking six classes or groups of treated guinea-pigs as given in STOCKARD and PAPANICOLAOU'S (1918) paper, the computation of the sex-ratio percentage with the probable error of each was made. These groups were then compared with a control group, the data being taken from the same paper. Table 2 gives the results obtained.

In five of STOCKARD'S classes the probable error is greater than the difference; in one the difference and its probable error are approximately equal; while all six classes taken together show a difference from the controls of 1.74 ± 2.46. In four of the groups the tests are less than the controls and in two the tests are greater than the controls, a random distribution of the high and low ratios exactly comparable with those in our data (see table 1 above).

In the light of this analysis it can no longer be maintained that alcoholic fumes have modified the sex ratio in guinea-pigs.

PEARL paid particular attention to the sex ratio in his fowls, and has presented the results in his characteristically clear and concise quantitative fashion. From his tables the following summary of his results has been made:

TABLE 3

A table showing the sex ratio in matings of treated×untreated; treated×treated; and untreated×untreated fowls. Data from PEARL.

	TOTAL CHICKS	SEX UNKNOWN	♂♂	♀♀	SEX RATIO
Treated×untreated	140	7	65	68	48.9 ± 2.8
Treated×treated	74	8	30	36	45.5 ± 4.1
Untreated×untreated	252	10	121	121	50.0 ± 2.2

PEARL'S conclusion based on the above data is that:

"Evidently these figures give no grounds for asserting that the relative proportions of the sexes produced are significantly different in the alcoholic and normal control series. If the treatment has had an influence on this character it has been so slight as not to be statistically discernible in samples of the size here dealt with."

BLUHM'S (1921) paper is the only one in the literature which seems definitely at variance with the results of STOCKARD, PEARL, and HANSON. BLUHM administered alcohol to male mice by subcutaneous injection. Her results can be most quickly presented in the form of a table:

TABLE 4

Data from BLUHM (1921).

	NUMBER OF LITTERS	NUMBER OF YOUNG	♂♂	♀♀
Controls	195	965	79.36	100
Treated	67		122.14	100

BLUHM suggests that there are two kinds of sperm and that the alcohol acts differently on them, the female-determining sperm losing something of their motility and hence increasing the number of males. This is not essentially different from the explanation offered by STOCKARD and quoted above.

BLUHM'S work, as PEARL says, should be repeated, since it is so far out of line with all other work. It will be noted (table 4) that the sex ratio of the controls is about as far from the normal in a "low" direction as the treated are in a "high" direction. There is also a great difference in the numbers of animals in the control and treated lines. In the light of these criticisms and the overwhelming evidence on the other side, this work can not be accepted until repeated and confirmed by BLUHM or others.

So far as I am aware, MACDOWELL, in his numerous papers on various aspects of the alcohol problem, does not treat the sex ratio at all.

CONCLUSION

The data of PEARL and HANSON, and the reanalyzed data of STOCKARD, taken together, indicate strongly that severe and prolonged administration of alcohol fumes does not have a differential effect upon the sex ratio in fowls, rats or guinea-pigs.

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MENDELIAN RATIOS AND THE GAMETOPHYTE GENERATION IN ANGIOSPERMS[1]

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TABLE OF CONTENTS

	PAGE
INTRODUCTORY	359
Manifestation of the waxy character in the gametophyte generation	360
Reciprocal combinations between heterozygous non-waxy and pure waxy stocks	362
Nature of the material and experimental procedure	362
Results with progenies R2 and R5	363
The question of heterogeneity in the distributions	365
A possible modifying factor	367
Effect of the gene for sugary on the distribution of non-waxy and waxy seeds after self-pollination	368
Cause of the deficiency of waxy seeds	369
The problem	370
Selective elimination of zygotes	370
Irregularities at sporogenesis and the possibility of selective action in the female gametophyte	371
Differential pollen-tube growth	371
Ratio of silks pollinated to seeds produced	374
Selective fertilization	375
Interpretation of heterogeneity	376
Attempts to modify the zygotic distribution by pollen treatments	377
Effect of ultra-violet light	377
Effect of desiccation	380
DISCUSSION	381
SUMMARY	385
LITERATURE CITED	387
APPENDIX—Tables 13 to 17	389

INTRODUCTORY

In the flowering plants an entire generation, the gametophyte, intervenes between reduction and fertilization. Yet the results of numerous studies in heredity in this group can be explained on the assumption that a random assortment of genetic factors enters into the formation of the zygotes. The inference is that the genes do not exert a differential effect in the development of the haploid generation. It seems quite clear now,

[1] Papers from the Department of Genetics, Agricultural Experiment Station, UNIVERSITY OF WISCONSIN, No. 51. Published with the approval of the Director of the Station.

however, that some exceptions to this rule occur; occasionally, at least, the distribution of gametes entering into fertilization is influenced by the events which transpire subsequent to reduction. These anomalous cases afford a point of approach to the general problem of the status of the gametophyte generation of angiosperms in Mendelian inheritance.

Studies on the inheritance of the waxy character in maize by COLLINS and KEMPTON (1911) and by KEMPTON (1919) show that a deficiency of waxy seeds, larger than can reasonably be ascribed to chance, occurs when individuals heterozygous for this character are self-pollinated. On finding that such plants produce two equally frequent sorts of pollen, the one kind staining bluish with iodine like that of the non-waxy parent and the other yellowish to reddish-brown like that of the waxy variety, BRINK and MACGILLIVRAY (1924) suggested that the deviation from expectation on the Mendelian basis was probably caused by a differential rate of development of the two classes of male gametophytes.

In the present investigations the microchemical nature of the female gametophytes arising from waxy hybrids was studied; and additional evidence concerning the ratio in which non-waxy and waxy seeds are formed after reciprocal crosses between the homozygous waxy type and the heterozygote has been obtained. A study of the effect of self-pollinating maize plants homozygous for the sugary or sweet factor and segregating for waxy yielded a significant body of data. A critical experiment to test out the hypothesis previously advanced that the deficiency of waxy grains is due to a lower rate of growth of waxy pollen tubes was performed. And finally, tests were made to determine to what extent the ratio of non-waxy and waxy seeds can be modified by exposing the pollen from heterozygous individuals to agencies whose effects might possibly be selective.

On the basis of the data available from pedigree-culture experiments and physiological studies, an attempt is made to define the status of the gametophyte generation of angiosperms in Mendelian inheritance.

MANIFESTATION OF THE WAXY CHARACTER IN THE GAMETOPHYTE GENERATION

DEMEREC (1924) and BRINK and MACGILLIVRAY (1924), independently, have shown that the pollen of the waxy race of maize may be distinguished from that of non-waxy varieties by staining with iodine; individuals heterozygous for the waxy factor produce two classes of pollen with equal frequency, corresponding, respectively, to the types formed by the two

parental stocks. It seemed of interest to determine if such a difference was shown likewise by the female gametophyte.

Ovaries from the ears on two heterozygous plants were collected and fixed in chromo-acetic and alcohol-formalin killing solutions. The material was imbedded in paraffin and microtome sections 10 μ thick were cut and mounted. After passing through xylol and alcohol solutions of decreasing concentrations, the slides were stained with iodine. Two solutions of the latter substance were used, a strong and a weak. The former contained 1.0 gm KI and 0.3 gm I to 100 cc of water, while the latter was made by diluting one part of the strong iodine solution with 3 parts of water. The slides were first placed for 2 or 3 minutes in the weak solution, then rinsed in water to remove the excess iodine. Microscopic examination at this point shows starch grains in the wall of the ovary staining a faint blue. If the grains in the embryo sac are starchy, they likewise appear blue after this treatment. In observing the color reactions, daylight is more satisfactory than artificial light.

If there are no blue-staining grains apparent in the embryo sac, the slide is then placed in the strong iodine solution. A few-seconds treatment is sufficient to cause any grains imbedded in the cytoplasm of the embryo sac to become differentiated. In fact, they stain so deeply that they appear black. The section is then treated with a few drops of a saturated solution of chloral hydrate. This swells the grains and causes the stain to fade out rapidly. With this treatment the starch in the ovary wall shows the characteristic blue reaction, whereas the granules in the embryo sac, if the latter is of the waxy type, stain a brownish-yellow.

The amount of starchy or waxy material in the embryo sac varies from an abundance of granules scattered in the cytoplasm at the micropylar end or clustered around the polar nuclei, to none.

Occasionally it was found that, even with the chloral hydrate treatment, it was impossible to distinguish clearly whether the reaction was of the non-waxy or the waxy type, due to the fact that the color of the granules was obscured by that of the yellow-staining cytoplasm in which they were imbedded.

The results of these observations are presented in table 1.

TABLE 1

Results of microchemical tests for non-waxy and waxy female gametophytes.

NUMBER OF NON-WAXY EMBRYO SACS	NUMBER OF WAXY EMBRYO SACS	NUMBER OF DOUBTFUL CASES	NUMBER OF EMBRYO SACS DEVOID OF GRANULES
25	20	5	12

These data show that the waxy gene produces essentially the same effect in the female gametophyte as in the pollen. Its action in the embryo sac, as we shall see later in this paper, is not fraught with the same genetic consequences, however, as in the case of the male gametophyte. Incidentally, it may be mentioned that the establishment of this difference provides evidence in addition to that afforded by the behavior in inheritance of endosperm characters that the female gametophyte of maize is not of the "lily" type; if all four megaspores survived, we should not be able to distinguish any waxy-type embryo sacs on hybrid plants on account of the dominance of the non-waxy factor. This is in agreement with the view of WEATHERWAX (1923) and opposed to that of MILLER (1919).

RECIPROCAL COMBINATIONS BETWEEN HETEROZYGOUS NON-WAXY AND PURE WAXY STOCKS

As mentioned above, the extensive tests made by COLLINS and KEMPTON (1911) and KEMPTON (1919) show clearly that a significant deficiency of waxy grains results when plants heterozygous for this character are selfed; it was also found that crosses of the heterozygote on the recessive waxy type resulted in a similar deviation from expectation, whereas the reciprocal combination produced a small, apparently inconsequential excess of waxy seeds. The deviations found in the two latter cases, however, were based on much smaller numbers than obtained in the first group. Since the definite establishment of a possible difference in reciprocal crosses is a matter of primary importance in seeking an explanation of the phenomenon, it seemed advisable to test these combinations further. Moreover, it is of considerable theoretical interest to know whether the selective action is manifested in matings when the pistillate parent is homozygous for waxy as well as when it carries the non-waxy factor.

Nature of the material and experimental procedure

A word of explanation is required in regard to the stocks employed in these tests. Pedigreed material from two ears originally designated for the experiments in question suffered an almost total loss through root rot (Gibberella?) during the very unfavorable spring of 1924. The corn was planted on May 13 and during the succeeding two weeks the mean soil temperature at a depth of 2 inches, as computed from thermographic records taken in a similarly exposed position adjacent to the breeding plot, was 13°C. Under these conditions corn grows very slowly; the root-rot organism, on the other hand, thrives and the incidence of disease may become very high. The immediate result in these particular progenies

grown from apparently healthy, but Gibberella-infected, seed, was a reduction in stand of 58 percent and the weakening of the surviving plants to such an extent that very few usable individuals developed. The only other stocks of the desired constitution with respect to the non-waxy genes available in sufficiently large numbers were two pairs of progenies derived from the non-waxy and the waxy seeds on two ears from open-pollinated waxy plants. The progeny grown from the waxy seeds on the first ear was designated R 2b and that from the heterozygous non-waxy, R 2a. The respective progenies obtained from the second ear were R 5a and R 5b.

It was not suspected at the time that genes other than the non-waxy allelomorphs might influence in a significant way the distribution of the two classes of seed. To facilitate obtaining a large number of ears, crosses were made between the groups R 2b and R 2a and also R 5a and R 5b without regard to the particular plants chosen as parents in the cross combinations. That is, the matings $W_x\ w_x$ ♀ × $w_x\ w_x$ ♂ and $w_x\ w_x$ ♀ × $W_x\ w_x$ ♂ were arranged on the assumption that, irrespective of their origin, the individuals within each of the types concerned were of equal value with respect to the factors involved in determining the proportion of non-waxy and waxy seeds. As will be evident when the results are presented, such a procedure is not calculated to furnish a complete solution of the genetic basis of the deviations encountered. The test we desired to make on a scale sufficient to establish the significance of small differences, however, was for a possible divergence in the results of crosses of the types $W_x w_x$ ♀ × $w_x w_x$ ♂ and $w_x w_x$ ♀ × $W_x w_x$ ♂. The facts obtained provide a definite answer to this problem but raise a further question regarding the effect of other variables not under control in this material. The latter point we shall deal with in due course.

Results with progenies R 2 and R 5

Since the results of the crosses within each of the two pairs of progenies R 2a, R 2b and R 5b, R 5a are very similar, they will be considered together. From combinations of the type $W_x w_x$ ♀ × $w_x w_x$ ♂, that is, where the heterozygote was used as the pistillate parent, 82 ears were obtained, bearing a total of 37,049 seeds. The numbers of non-waxy and waxy grains on each ear are given in tables 13 and 14. Table 2 contains a summary of the data. The mean percentage of waxy seeds in the whole group differs from expectation on the Mendelian basis by only 0.18 percent, an amount well within the limits of random sampling. Inspection of the frequencies in the 86 subgroups listed in tables 13 and 14 (appendix) shows that 76

TABLE 2

Summarized results of combinations of the type $W_x\ w_x$ ♀ × $w_x\ w_x$ ♂, *in progenies R 2a, R 2b and R 5b, R 5a.*

CROSS	NUMBER OF EARS	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION	DEV. / P. E.
		Total	Non-waxy	Waxy			
R2a×R2b	48	21,436	10,759	10,677	49.81±0.23	−0.19	0.83
R5b×R5a	34	15,613	7,833	7,780	49.83±0.27	−0.17	0.63
Totals	82	37,049	18,592	18,457	49.82±0.17	−0.18	1.03

deviate from the expected proportions by amounts less than three times the respective probable errors. In the six remaining ears, two of which show an excess and four a deficiency of individuals in the waxy class, the deviations, in four cases at least, are too large to be satisfactorily accounted for as purely chance events. Except for these variations, however, the data are in close agreement with Mendelian expectation.

A different situation is presented when we consider the results of the reciprocal cross, that is, where the heterozygote was used as the staminate parent. A group of 75 ears was obtained, bearing a total of 33,020 grains, 48.25 percent of which were waxy. The data are summarized in table 3.

TABLE 3

Summarized results of combinations of the type $w_x\ w_x$ ♀ × $W_x\ w_x$ ♂ *in progenies R 2b, R 2a and R 5a, R 5b.*

CROSS	NUMBER OF EARS	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION	DEV. / P. E.
		Total	Non-waxy	Waxy			
R 2b×R 2a	33	14,773	7,628	7,145	48.36±0.28	−1.64	5.85
R 5a×R 5b	42	18,247	9,458	8,789	48.17±0.25	−1.83	7.32
Totals	75	33,020	17,086	15,934	48.25±0.19	−1.75	9.41

The net minus deviation in the waxy class, 1.75 percent, is over 9 times its probable error and the odds against its being due to chance alone are inconceivably great. Moreover, 20 of the 75 ears listed in tables 15 and 16 show variations from the expected 1 : 1 ratio greater than 3 times the respective probable errors. Of these 20 subgroups, 17 have fewer waxy individuals than Mendelian theory calls for and 3 show an excess. That there is a well defined tendency towards a deficiency of waxy individuals is shown by the fact that on two-thirds of the ears less than 50 percent of

the seeds were of this sort. It should be noted, however, that 3 ears, each bearing more than 500 grains, show significant deviations in the opposite direction. This leads us to a consideration of the possible heterogeneity in this group.

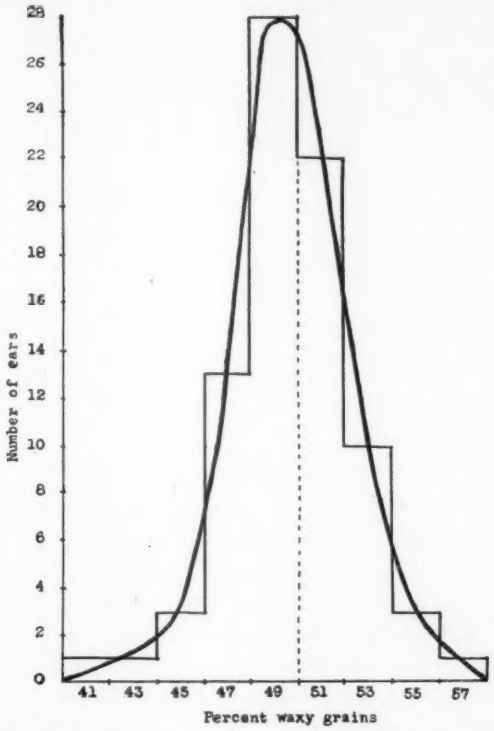

FIGURE 1.—Distribution of ears with respect to percentage of waxy grains following the combination of the type $W_z\, w_z$ ♀ × $w_z\, w_z$ ♂ with progenies R 2a, R 2b and R 5b, R 5a.

The question of heterogeneity in the distributions

In table 4 and in figures 1 and 2 the frequency distributions of ears with respect to percentage of waxy grains in the two groups resulting from reciprocal combinations is shown. Graphic representation of these populations reveals obvious differences in their character. As represented in figure 1 the ears resulting from crosses of the type $W_z\, w_z$ ♀ × $w_z\, w_z$ ♂ are symmetrically distributed about a mean at approximately 50 percent and

TABLE 4

Frequency distribution of ears in reciprocal crosses, $W_x\ w_x$ ♀ × $w_x\ w_x$ ♂ and $w_x\ w_x$ ♀ × $W_x\ w_x$ ♂. Combined data from R 2a×R 2b, R 5b×R 5a and R 2b×R 2a, R 5a×R 5b.

	PERCENT WAXY SEEDS												TOTAL	TRUE MEAN	STANDARD DEVIATION IN PERCENT
	41	43	45	47	49	51	53	55	57	59	61	63			
$W_x\ w_x$ ♀ × $w_x\ w_x$ ♂	1	1	3	13	28	22	10	3	1				82	49.82±.17	2.67±.14
$w_x\ w_x$ ♀ × $W_x\ w_x$ ♂	2	8	12	14	15	15	4	2	1	1	0	1	75	48.25±.19	3.98±.22
Differences														1.57±.25	1.31±.26

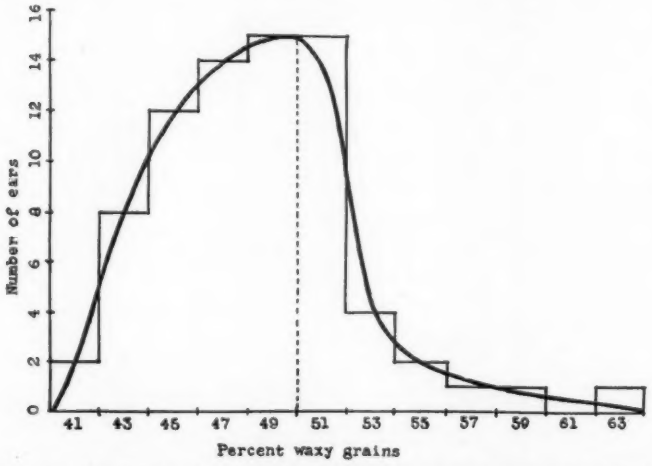

FIGURE 2.—Distribution of ears with respect to percentage of waxy grains following the combination of the type $w_x\ w_x$ ♀ × $W_x\ w_x$ ♂ with progenies R 2b, R 2a and R 5a, R 5b

show comparatively low variability. On the other hand, the ears resulting from the application of pollen from heterozygous plants to pure waxy individuals form a minus-skew curve with relatively greater "spread" as shown in figure 2. YULE (1914) has proposed a method to determine if the subgroups comprising a population can reasonably be considered as random samples of the whole material.[2] This method involves a com-

[2] If p is the probability of obtaining one of the two allelomorphic characters and q the chance of obtaining the other, the expected standard deviation of the proportion in a group of samples of different sizes is equal to $\sqrt{pqr}$, where r is equal to the reciprocal of the harmonic mean of the numbers in the samples.

parison of the observed standard deviation with that expected if the variates form a homogeneous group normally distributed around a common mean.

These values have been calculated for the two populations in question. The observed standard deviation of the group of 82 ears resulting from crosses of the type $W_x\ w_x\ ♀ \times w_x\ w_x\ ♂$ is $2.64 \pm .14$ percent; the calculated value is 2.44 percent. The relatively small difference, amounting to $0.23 \pm .14$ percent, might well be due to chance. There is little, if any, indication, therefore, of heterogeneity in this population.

Determination of the corresponding values for the group of 75 ears resulting from the reciprocal cross, $w_x\ w_x\ ♀ \times W_x\ w_x\ ♂$, bears out the idea that the curves in figures 1 and 2 differ in an essential respect. The observed standard deviation in this case is $3.98 \pm .22$ percent, whereas the expected value is 2.49 percent. The difference, 1.49 percent, is over 6 times its probable error and would be expected to occur but once in many thousand trials as a result of pure chance. We may confidently conclude, therefore, that the distributions from these ears, resulting from the use of pollen from individuals segregating for the waxy character, do not form a homogeneous group. The variability is too high to result from the operation of chance alone.

A possible modifying factor

In a stock obtained from Doctor A. C. FRASER, CORNELL UNIVERSITY, of different origin from the above, further evidence has been obtained that the distribution of non-waxy and waxy grains on ears resulting from the use of pollen from the heterozygote can not be fully accounted for on the basis of the action of the non-waxy-waxy allelomorphs alone. It is planned to study this material intensively another year, but it may be worth while to indicate the nature of the evidence at present available.

The seeds from a single ear resulting from a cross of the type $A\ C\ R\ I\widehat{W_x}.\ \widehat{iw_x} \times A\ C\ R\ \widehat{iw_x}.\ \widehat{iw_x}$ were separated into the two parental classes, colorless non-waxy and colored waxy, and the two crossover groups, colorless waxy and colored non-waxy. Each of these lots was grown separately and reciprocal crosses were made between the two non-crossover stocks and likewise between the crossover progenies. It was interesting to find that the two sets of matings of the type $w_x\ w_x\ ♀ \times W_x\ w_x\ ♂$ did not yield the same result. The combination between the two non-crossover progenies produced a probably significant excess of waxy seeds, whereas, mating the two crossover groups gave a relatively large and clearly significant deficiency. Furthermore, in the group of high-waxy ears, containing

members bearing as many as 63 percent waxy grains, the excess of individuals of this sort came entirely in the non-crossover class of seed $(\widehat{i\ w_z})$.

Since data on but 25 plants are available at present, one can only speculate as to the cause of this peculiar distribution, but the facts indicate clearly that in explaining the action of the non-waxy-waxy pair, other genes must be taken into consideration.

EFFECT OF THE GENE FOR SUGARY ON THE DISTRIBUTION OF NON-WAXY AND WAXY SEEDS AFTER SELF-POLLINATION

To test for a possible influence of the gene for sugary on the non-waxy-waxy ratio a progeny of 63 plants designated R 11 was grown from sugary seed for which I am indebted to Mr. M. T. MYERS of OHIO STATE UNIVERSITY, resulting from a single cross, $s_u\ s_u\ W_z\ W_z \times S_u\ s_u\ W_z\ w_z$. On self-pollinating these plants, the 25 that were found to be segregating for waxy yielded 31 ears. The non-waxy and waxy grains were classified on these by means of the iodine test. Each kernel was split lengthwise through the embryo and one portion immersed in a solution of iodine and potassium iodide for a few minutes, the excess stain being then thoroughly washed off with water. With this treatment, the waxy seeds stain a dull orange, and the non-waxy seeds a dark blue, shading to reddish in certain parts of the broken surface. These gross differences can be readily checked by mounting a little endosperm material in dilute iodine and observing it under a compound microscope, where the treated non-waxy and waxy granules regularly appear blue and red, respectively. The color reaction of the small "soft starch" area in the split grain is a reliable index of type, but if overstaining is avoided the appearance of the whole cut surface differs in the two classes of seeds. We have frequently checked our determinations with the microscope and feel confident that a high degree of accuracy was attained.

The ratios of non-waxy and waxy seeds on the individual ears in the sugary progeny, R 11, are listed in table 17. The results are thrown into the form of a frequency distribution in table 6. Where a single plant bore two ears, the percentages of waxy seeds on these have been averaged. So far as it is possible to judge from this small population, the plants appear to form a homogeneous group with respect to the factors affecting the proportion of non-waxy and waxy seeds; the mode is well defined at the 18 percent class and the variability is relatively low.

It will be noted from the general summary given in table 5 that the net percentage of waxy grains is far below that expected. The deficiency amounts to 6.16 percent, a deviation over 23 times its probable error.

The odds are overwhelming against the difference being due to chance alone. Each subgroup in this population shows a deficiency of waxy seeds and in 25 of the 31 cases the deviations are greater than three times their respective probable errors. Data concerning the ratio of non-waxy and waxy grains on non-sugary ears of the same breeding as the above are not available but can be obtained in another season.

While the possibility remains that some other factor brought in by this stock is responsible, there is considerable justification for the conclusion that the gene for sugary greatly increases the disparity between the observed proportion of non-waxy and waxy seeds and that expected on a chance basis.

TABLE 5

Summary of results of self-pollinating the plants segregating for waxy in the homozygous sugary progeny, R 11.

NUMBER OF EARS	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN PERCENT IN WAXY CLASS	DEV. / P. E.
	Total	Non-waxy	Waxy			
31	12,264	9953	2311	18.84± .26	−6.16	23.69

Odds = several million to 1

TABLE 6

Frequency distribution of plants in the homozygous sugary progeny, R 11, with respect to percentage of waxy grains.

Class values (percent)	14	16	18	20	22	24	TOTAL
Frequency	1	4	12	6	1	1	25

CAUSE OF THE DEFICIENCY OF WAXY SEEDS

The problem

Supplementing and extending the findings of COLLINS and KEMPTON (1911) and KEMPTON (1919), the facts presented above show that, on the average, when maize plants segregating for the waxy character are used as staminate parents in combination with the recessive type a clearly significant deficiency of waxy seeds results. It is equally certain that in the cross $W_x w_x$ ♀ × $w_x w_x$ ♂, that is, where the heterozygote is employed as the pistillate parent, the deviation from expectation on the Mendelian basis is no greater than would be frequently expected as the result of pure chance. On self-pollinating individuals homozygous for sugary and heterozygous for waxy the ratio of non-waxy to waxy grains produced was found

to be 4.4 : 1. In the material studied, the variability in the proportion of non-waxy and waxy grains on the individual ears resulting from combinations of the type $w_x w_x$ ♀ × $W_x w_x$ ♂ was shown to be greater than can be attributed to the operation of chance alone. In addition to the differential occasioned by the waxy factor other variables must, therefore, influence the ratio in which the two classes of seeds are formed. These, briefly, are the facts we have to account for.

The interest in this problem lies not alone in arriving at an interpretation of the observed facts in terms of the distribution of genetic factors, but also in discovering when and how the germinal elements involved bring about their differential effect. Indeed, some definite knowledge of the latter phase of the matter is prerequisite to an interpretation of the hereditary relations. A mathematical description of the breeding facts, to have predictive value, must take into account the essential conditions limiting the operation of the hereditary units postulated. It is true that great progress in genetics has been made without much insight into the conditions involved in the manifestation of a character, but this is only because the differential effects dealt with have been, for the most part, of such a nature that they were distinguishable under a relatively wide variety of environmental circumstances. Such characters have been invaluable in establishing laws of heredity which, we have good reason to believe, are of general validity, but the usefulness of these rules in practice is often much restricted through our ignorance of the accessory conditions affecting the developmental processes. When the "latitude of genetic indetermination" is relatively wide, our problem becomes as much a physiological as a genetic one. In the present case, where modification of a simple Mendelian ratio is the end result, it is necessary, in order to define the problem, to discover the stage at which the usual course of events is altered; in forming a judgment of the significance of the phenomenon for genetic theory and in establishing a basis for prediction a knowledge of the nature of the changes involved is requisite also. Attention has been given these matters.

Selective elimination of zygotes

If the deficiency of waxy individuals were due to a tendency for this type to be eliminated during the post-fertilization stage, we should expect to find the condition associated with partially filled ears. Such is not the case; ears showing a low ratio of waxy grains are as fruitful as normal ones. It would be expected also that if elimination during the embryonic stage were the true explanation, reciprocal crosses would be alike; the evidence that they differ, however, is indubitable.

Irregularities at sporogenesis and the possibility of selective action in the female gametophyte

It is conceivable also that the modification of the ratio might be caused by irregularities at the reduction divisions resulting in the production of fewer waxy spores than expected. Numerical data (DEMEREC 1924, BRINK and MACGILLIVRAY 1924) are available, however, which prove that segregating plants produce equal numbers of non-waxy and waxy pollen grains. Furthermore, breeding evidence has been obtained showing that in the backcross, $W_x\ w_x\ ♀ \times w_x\ w_x\ ♂$, the ratio of non-waxy and waxy seeds is as close to Mendelian proportions as can reasonably be expected. This demonstrates that the 1 : 1 proportion is not disturbed at megasporogenesis.

If our knowledge of the irregularity in the non-waxy-waxy ratio were limited to the results obtained on self-pollinating the heterozygote, we might suspect that the female gametophyte was involved. It might be supposed, for example, that there is a greater tendency for the waxy pollen tubes to enter the non-waxy type rather than the waxy type of ovule; or, that there is some discrimination against the fusion of male and female waxy gametes. It will be shown in the following sections that both these assumptions are not only unnecessary but unwarranted.

Differential pollen-tube growth

An experiment was carried out during the past season to test out the hypothesis advanced by BRINK and MACGILLIVRAY (1924) that the deficiency of waxy grains is due to differential pollen-tube growth. The progeny, designated as R 12, used in these tests, was derived from a single cross between a homozygous waxy stock self-pollinated once and a six-times-selfed strain of a non-waxy variety commonly known as Burr's White Dent. As would be expected in view of the nature of their parents, the plants showed a high degree of uniformity in their gross vegetative characters; they tillered freely and produced a large number of rather small-sized ears.

If a difference in rate of growth of non-waxy and waxy pollen tubes is responsible for the deficiency of waxy seeds, the disparity in the proportion of the two zygotic classes should be augmented if the distance the tubes must travel before they reach the embryo sac is increased. This test was made by self-pollinating a series of ears whose silks had been permitted to grow to an unusual length under the protection of paper bags. To localize definitely the pollinated area to the outer ends, the silks were

wrapped lightly with about three turns of paper fastened in place with small elastic bands. The tips of the silks were then trimmed off so that they protruded about one inch beyond the edge of the wrapper. This exposed portion was then thoroughly covered with freshly collected pollen. In a series of 22 ears prepared in this fashion the distance from the outer edge of the paper band to the tip of the ear ranged from 9 to 13 inches and averaged nearly 11 inches; and the distance from the same point to the butt of the ear varied from 12 to 18 inches with a mean value of about 15 inches.

Of the 22 ears treated in this test, 15 set grain. Of those which failed to produce any kernels, one was a first ear, that is, was the uppermost ear on the stalk, the one usually having the advantage in development; four were second ears and two were third ears on the respective stalks which bore them. Plants in this progeny very seldom matured third ears but open-pollinated second ears, even on rather small stalks, were freely produced. It is impossible to account for many of the failures which occur in artificially pollinated maize. Bagging the ear retards development and may often be a determining factor. In the present case the long-styled condition greatly reduced the yield of grain on fruitful ears and may have been responsible for some of the total failures. This possibility is discussed below.

In classifying this material as well as that obtained in the experiments involving pollen treatments discussed below, the seeds were shelled off the cob, a small area of the endosperm of each grain exposed with a sharp knife and stained with an aqueous solution of iodine and potassium iodide. Waxy seeds thus treated color a reddish-brown, whereas non-waxy grains stain blue. This procedure is not always necessary in order to distinguish between non-waxy and waxy grains but when a considerable amount of soft starch is present it affords a uniformly certain means of separating the two classes.

Since all the individuals used in this experiment were heterozygous for waxy, as was determined directly by examination of the pollen of each plant in an iodine solution (cf. DEMEREC 1924, BRINK and MACGILLIVRAY 1924) and self-pollination was practiced, the expectation on the Mendelian basis is that the two classes of seeds, non-waxy and waxy, will be produced in a ratio of three to one. The results obtained are presented in table 7. It will be noted that 12 ears show a deficiency of waxy kernels, 3 ears have an excess, and that in the total population of 1263 individuals there resulted a net deficit of waxy seeds amounting to 57.75. This deviation from expec-

TABLE 7

Distribution of non-waxy and waxy seeds on the long-styled series of selfed ears in progeny R 12 together with the number of silks pollinated in each case.

EAR NUMBER	TOTAL SEEDS	NUMBER OF SILKS POLLINATED	PERCENTAGE EFFECTIVE POLLINATIONS	NON-WAXY SEEDS	WAXY SEEDS	EXPECTED WAXY	DEVIATION IN WAXY CLASS
8–2	13	342	3.8	11	2	3.25	− 1.25
8–3	11	247	4.4	10	1	2.75	− 1.75
17–2	28	281	10.0	20	8	7.00	+ 1.00
19–3	32	....	....	22	10	8.00	+ 2.00
19–4	93	244	38.1	72	21	23.25	− 2.25
23–2	21	292	7.2	20	1	5.25	− 4.25
38–1	129	325	39.7	105	24	32.25	− 8.25
42–1	190	319	59.6	146	44	47.50	− 3.50
43–2	8	328	2.4	7	1	2.00	− 1.00
50–2	98	371	26.4	80	18	24.50	− 6.50
53–3	220	237	92.8	180	40	55.00	−15.00
54–1	104	348	29.9	77	27	26.00	+ 1.00
54–2	26	304	8.5	20	6	6.50	− 0.50
59–1	88	346	25.4	73	15	22.00	− 7.00
60–2	202	266	75.9	162	40	50.50	−10.50
Totals	1263	4250	29.7	1005	258	315.75	−57.75

$\frac{\text{Dev.}}{\text{P.E.}} = \frac{57.75}{10.38} = 5.56$. Odds = ca 6000 : 1. Percentage of waxy seeds = $20.43 \pm .82$.

tation on the monohybrid basis is 5.56 times its probable error and the odds against its being due to chance alone are of the order of 6000 to 1.

In order to decide whether increasing style length has, *per se*, altered the zygotic frequencies, it is necessary to compare the above results with those obtained in the check series from the same progeny. This group consisted of 19 ears whose silks were trimmed off to a point about 3 inches above the tip of the young ear. The ears operated upon were in various stages of development; in a few instances the silks before transecting were as long as those in the long-styled series; but in the majority of cases the styles were relatively young and short. It is not known at present whether this age difference is of any significance. These ears were thoroughly dusted with freshly collected pollen and 13 of them developed to maturity.

The proportions of non-waxy and waxy seeds are given in table 8. With respect to the waxy class, plus deviations are shown in 3 cases, minus deviations in 9 cases, and no deviation in one. In the total population of 3569 individuals there is a net deficit in the waxy class of 37.25 ± 17.45

TABLE 8

The distribution of non-waxy and waxy seeds on the check series of selfed ears in progeny R 12 and the approximate number of silks pollinated.

EAR NUMBER	TOTAL SEEDS	NUMBER OF SILKS POLLINATED	PERCENTAGE EFFECTIVE POLLINATIONS	NON-WAXY SEEDS	WAXY SEEDS	EXPECTED WAXY	DEVIATION IN WAXY CLASS
4-1	321	485	66.2	239	82	80.25	+ 1.75
6-2	151	290	52.1	119	32	37.75	− 5.75
7-2	229	348	65.8	186	43	57.25	−14.25
8-4	245	217*	....	186	59	61.25	− 2.25
8-5	240	467	51.4	185	55	60.00	− 5.00
10-2	392	441	88.9	294	98	98.00	0.00
20-3	246	...	....	183	63	61.50	+ 1.50
20-4	272	338	80.5	206	66	68.00	− 2.00
20-5	330	337	97.9	252	78	82.50	− 4.50
32-4	313	464	67.0	230	83	78.25	+ 4.75
39-3	172	466	36.9	135	37	43.00	− 6.00
40-1	318	463	68.7	241	77	79.50	− 2.50
42-2	340	373	91.1	258	82	85.00	− 3.00
Totals	3569	4472	79.8	2714	855	892.25	−37.25

$\frac{\text{Dev.}}{\text{P.E.}} = \frac{37.25}{17.45} = 2.13$. Odds = 5.4 : 1. Percentage waxy seeds = 23.96 ± .49.

* This is evidently a mistake and this ear is not included in figuring the percentage of effective pollinations.

seeds. Such a deviation from the exact Mendelian proportion is expected about once in 6 trials, on the average, purely as a matter of chance.

The percentage of waxy grains produced on the ears with long silks is 20.43 ± .82 as compared with 23.96 ± .49 percent on those of the check group. The difference, 3.53 ± .96 percent, appears to be statistically significant, since the odds against its being due merely to fluctuations of sampling are about 75 to 1. It seems justifiable to conclude, therefore, that increasing the length of the style actually increases the disproportion of non-waxy and waxy seeds and that differential pollen-tube growth is responsible for the deficiency of waxy seeds that commonly results when heterozygous waxy plants are used as staminate parents under the conditions ordinarily obtaining in genetic experiments with maize.

Ratio of silks pollinated to seeds produced

A result in the long-styled series which was not anticipated was the reduction in the number of seeds formed far below expectation on the basis of the number of silks pollinated. When it was found, two weeks

after pollination, that several of the members in this group were developing very few grains, the tips of the silks on each ear were cut off and counted in the laboratory. As the elastic bands on the wrappers prevented the emergence of any additional silks during the interval, the values given in table 7, barring relatively small errors in counting, perhaps, actually represent the number of silks pollinated. In three cases, only, does the percentage of effective pollinations exceed 40. On the average about three and one-third silks were pollinated for each grain produced. At the same time (two weeks after pollination) the number of silks on each of the check ears was counted also (table 8). In this group, however, the values obtained do not represent as accurate an estimate of the number of silks actually pollinated as in the former case, since the number that grew out in the interval were, perforce, included also. This would tend to increase the ratio of silks pollinated to grains produced. In spite of this error, the data show an average of 80 percent effective pollinations or over two and one-half times the proportion found in the case of the ears with long styles.

Among the explanations that might be offered to account for these facts. one, at least, is worthy of consideration as a possible factor in modifying the non-waxy ratio. Perhaps the length to which maize pollen tubes may grow is determinate and the limit in this stock was closely approached in the long-styled series. This is merely a suggestion, but if it were true, then it would appear that non-waxy pollen tubes not only grow faster, but they also attain greater average length than their waxy competitors.

Selective fertilization

Selective fertilization has been invoked from time to time as an explanation of aberrant genetic ratios. The term has been applied to cases in seed plants very similar to the one at present being considered. Its use, however, carries an implication that probably is not intended. Fertilization comprehends only those processes involved in the actual fusion of the gametes. Selective fertilization denotes discrimination at this stage in favor of certain types of gametes. The evidence brought forward in maize with reference to the waxy case lends no support to the view that the cause of the disturbance in the zygotic ratio is a preferential fusion of gametes. On the other hand, the argument that it is due to differential pollen-tube growth is convincing. Pollen-tube growth, of course, is a process separate and distinct from fertilization. Differential pollen-tube growth leads to a modification in the ratio in which the gametes are

presented at fertilization, but the resulting change in the zygotic ratio is no more to be attributed to selective fertilization than is the difference in the number of dominants found when we mate a recessive with the allelomorphic homozygote instead of with the heterozygote.

Selective fertilization, if it were demonstrated, would be a matter of primary importance for general genetic theory. If the phenomenon exists, we might look for it in any form reproducing sexually. On the other hand, differential pollen-tube growth is a special case, possible, of course, in but one group of organisms, the seed plants. It seems quite certain now that it occurs, but it is not to be confused with selective fertilization.

Interpretation of heterogeneity

A satisfactory interpretation of the heterogeneity shown by the populations of ears resulting from the application of pollen from plants heterozygous for waxy to the recessive type and the similar diversity which KEMPTON (1919) found among the offspring of self-pollinated waxy hybrids can not be given, perhaps, with the data now at hand. It would appear, however, that this variation has a genetic basis, although the mode of action of the modifying genes may not be readily defined. The results with the stock obtained from Doctor FRASER of CORNELL, as discussed above, suggest that in this material a factor in addition to the non-waxy-waxy allelomorphs is operating, but further work is necessary before any definite conclusion may be drawn.

In a former paper (BRINK 1924) the view was expressed that the carbon supply may be the most important element in the nutrition of the male gametophyte. In a synthetic medium consisting of 7 percent c.p. sucrose and 0.01 M $CaCl_2$ in distilled water the writer has secured pollen tubes of *Vinca minor* whose length closely approaches that of the style in this species. It appears from this result that the microgametophyte is largely independent of external sources of nitrogen and requires only a sugar as a source of building material and energy. It seems highly probable, at least, that the carbohydrate supply is the predominating nutritive factor in development. Differential pollen-tube growth, therefore, might well be due to modifications in carbohydrate metabolism. Investigations at present being carried on in our laboratory show that the passage of the pollen tube through the style of maize occasions measurable changes in the sugar reserves. We have found, moreover, that comparable lots of starchy and waxy pollen effect certain carbohydrate transformations at quite different rates. Perhaps this is the main effect of the waxy gene.

In seeking an explanation of the heterogeneity found we might suppose that the waxy factor induces a condition of instability in the carbohydrate system and opens the way for the modification of such processes as pollen-tube growth by other genes ordinarily inoperative in the presence of the allelomorph of waxy. The number of such modifying factors and the extent of their action is not known. It has been shown, however, that the gene for sugary disturbs the ratio very profoundly. Sugary, in itself, causes important changes in carbohydrate metabolism and the fact that it modifies the distribution in question affords substantial support to our hypothesis. In the stocks in which heterogeneity was found, however, factors other than sugary must be at work.

ATTEMPTS TO MODIFY THE ZYGOTIC DISTRIBUTION BY POLLEN TREATMENTS

Effect of ultra-violet light

The iodine test shows that the reserve materials in non-waxy and waxy pollen are different. While the chemical nature of this difference is not known, investigations in progress in our laboratory show that the reddish-brown-staining material in the pollen and endosperm of the waxy race is not a dextrin, since it is insoluble in water and in 10 percent alcohol. This reserve material is organized in granular form like typical starch; in quantitative chemical analyses it separates out in the starch fraction; and thus far we have found no means of differentiating it from typical starch except that it stains reddish-brown with iodine.

It was thought, however, that this difference in reserve materials might be correlated with a modification in kind or amount of enzymes. Ultra-violet light is known to be destructive to enzymes and an attempt was made to determine if it would exert a differential action on the two classes of pollen, thus causing a further deficiency in the waxy class of seed.

Progeny R 12, the same as used in the tests on effect of increasing style length, was employed in these experiments. Pollen from a number of plants was exposed to the rays from a quartz mercury vapor lamp for periods of 15, 30 and 45 minutes, it having been determined by laboratory tests that these dosages were not lethal. The pollen was then promptly applied to the plants from which it came. The lamp was operated on a voltage of 40 to 50, and 4 amperes, and the pollen exposed in a thin layer in open Petri dishes 14 inches below the arc. The heating effects were reduced by an electric fan so that the maximum temperature recorded in the Petri dishes was 32°C. This temperature would probably not cause injury.

Six well-filled ears were obtained in 6 trials with pollen irradiated for 15 minutes. The distributions of non-waxy and waxy grains obtained are given in table 9. It will be noted that a deficiency of waxy individuals resulted in each of the 6 cases and that the net minus deviation, 34.75, is 2.91 times its probable error, giving odds of about 19 to 1 against this departure from Mendelian expectation being due to chance alone. The percentage of waxy seeds on this set of ears is $22.93 \pm .71$ or $1.03 \pm .86$ percent less than on the checks. It can not be concluded on the basis of these data that irradiation with ultra-violet light for 15 minutes has occasioned a further decrease in the proportion of waxy zygotes.

TABLE 9

Distribution of non-waxy and waxy seeds on the ears in progeny R 12 resulting from the application of pollen irradiated with ultra-violet light for 15 minutes.

EAR NUMBER	TOTAL SEEDS	NON-WAXY SEEDS	WAXY SEEDS	EXPECTED WAXY	DEVIATION
27–2	248	190	58	62.00	− 4.00
53–1	211	163	48	52.75	− 4.75
61–1	301	231	70	75.25	− 5.25
62–1	302	236	66	75.50	− 9.50
63–1	303	229	74	75.75	− 1.75
64–1	310	242	68	77.50	− 9.50
Totals	1675	1291	384	418.75	−34.75

$\frac{\text{Dev.}}{\text{P.E.}} = \frac{34.75}{11.95} = 2.91$. Odds = ca 19 : 1. Percentage waxy seeds = $22.93 \pm .71$.

Pollen from eight plants was irradiated for 30 minutes and 7 ears were obtained as a result of its application. The counts on these ears are given in table 10. Comparing the observed results with the Mendelian expectation we find minus deviations in six cases and a plus variation in 1 case. There is a net minus deviation in the waxy class of 30.25 ± 12.10 seeds. This departure from expectation might well be due to chance.

From 5 attempts made with pollen irradiated with ultra-violet light for 45 minutes, 4 ears were secured. As shown in table 11, in two cases the proportion of waxy seeds was in excess and in the remaining two in defect of expectation on the monohybrid basis. The net minus deviation of waxy seeds in the total population of 869 is 8.25 ± 8.61 and might be expected to occur in about one-half the trials as a matter of chance.

TABLE 10

Distribution of non-waxy and waxy seeds on the ears in progeny R 12 resulting from the application of pollen irradiated with ultra-violet light for 30 minutes.

EAR NUMBER	TOTAL SEEDS	NON-WAXY SEEDS	WAXY SEEDS	EXPECTED WAXY	DEVIATION
12–1	250	198	52	62.50	−10.50
35–1	103	82	21	25.75	− 4.75
37–1	229	168	61	57.25	+ 3.75
39–1	273	214	59	68.25	− 9.25
41–1	239	180	59	59.75	− 0.75
49–1	360	277	83	90.00	− 7.00
51–1	263	199	64	65.75	− 1.75
Totals	1717	1318	399	429.25	−30.25

$\frac{\text{Dev.}}{\text{P.E.}} = \frac{30.25}{12.10} = 2.50$. Percentage waxy seeds = 23.24 ± .70.

TABLE 11

Distribution of non-waxy and waxy seeds on the ears in progeny R 12 resulting from the application of pollen irradiated with ultra-violet light for 45 minutes.

EAR NUMBER	TOTAL SEEDS	NON-WAXY SEEDS	WAXY SEEDS	EXPECTED WAXY	DEVIATION
11–1	325	259	66	81.25	−15.25
21–1	99	77	22	24.75	− 2.75
21–2	138	98	40	34.50	+ 5.50
52–2	307	226	81	76.75	+ 4.25
Totals	869	660	209	217.25	− 8.25

$\frac{\text{Dev.}}{\text{P.E.}} = \frac{8.25}{8.61} = .95$. Odds = ca 1 : 1. Percentage waxy = 24.05 ± .99.

If the results of the three irradiation experiments are combined it is found that the proportion of waxy individuals is in defect of expectation on the Mendelian basis by 73.25 ± 19.06 seeds. The odds are about 100 to 1 that this is a significant difference. The ears resulting from the application of irradiated pollen, however, show only 0.67 percent fewer waxy grains than the checks, a difference too small to justify the conclusion that this form of treatment exercised any differential action on the two classes of pollen. Possibly the heavy pollen-grain walls were not penetrated by the rays from the lamp.

Effect of desiccation

As the work of KNOWLTON (1922) clearly shows, maize pollen is very susceptive to desiccation. In the course of the present investigations experiments were arranged to determine if non-waxy and waxy pollen reacted differently to this factor. The 41 trials made included storage of the pollen in desiccators over concentrations of sulfuric acid giving relative humidities, according to the tables of WILSON (1921), of 35 percent and 65 percent, for periods ranging from 24 to 72 hours; storage over $CaCl_2$ at room temperature for periods of 12, 24, 48 and 72 hours and also exposure to the drying effects of $CaCl_2$ for 1 to 3 hours at a temperature of 40°C. In all of these experiments but two, the treatments given caused a complete or nearly complete inactivation of the pollen.

From the pollen samples exposed to the drying effect of $CaCl_2$ at room temperature for 12 hours, 4 ears were obtained in 6 trials, bearing the small total of 71 grains, 13 of which were waxy. The evidence is too meager to justify discussion of the effect of this treatment. The results of the tests with pollen desiccated for 1 to 3 hours over $CaCl_2$ at 40°C are more encouraging. The data are presented in table 12. Four ears show a deficiency of waxy seeds and two show an excess, and the net deviation in this class

TABLE 12

Distribution of non-waxy and waxy seeds on the ears in progeny R 12 resulting from the application of pollen dried over $CaCl_2$ at 40°C for 1 to 3 hours.

EAR NUMBER	TOTAL SEEDS	NON-WAXY SEEDS	WAXY SEEDS	EXPECTED WAXY	DEVIATION
9–1*	38	28	10	9.50	+ 0.50
20–1†	214	172	42	53.50	−12.50
24–1*	333	264	69	83.25	−14.25
36–1*	289	232	57	72.25	−15.25
48–1*	46	31	15	11.50	+ 3.50
58–1†	19	17	2	4.75	− 2.75
Totals	939	744	195	234.75	−39.45

$\frac{\text{Dev.}}{\text{P.E.}} = \frac{39.75}{8.94} = 4.44$. Odds = ca 400 : 1. Percentage waxy = $20.77 \pm .95$.

* Treated for 1 hour.

† Treated for 3 hours.

is 39.75 ± 8.94 grains. A chance deviation from Mendelian proportions of this magnitude is expected only about once in 400 trials. Moreover, as compared with the check series, this group of ears shows 3.19 ± 1.07 per-

cent fewer waxy seeds. The difference is 2.97 times its probable error and is possibly significant.

While one can not be sure, in view of the statistical relations shown, that any form of pollen treatment has had a differential action, it seems rather probable that exposure to $CaCl_2$ for 1 to 3 hours at 40°C was more injurious to the waxy pollen than to the non-waxy.

DISCUSSION

The numerical ratios in cases of simple Mendelian inheritance are the result of the chance distribution of the genes at two critical stages in the life cycle, namely, reduction and fertilization. Experimental proof (CAROTHERS 1917) has been furnished that the bivalent chromosomes, bearers of allelomorphic genes, assort by chance during reduction; random fertilization is inferred from the character of the zygotic distribution. Recombination in Mendelian proportions is conditional upon the absence of any selective process between reduction and fertilization.

In animals the gametes are the immediate products of reduction and, generally speaking, constitute the only $1n$ cells in the life cycle. The situation in plants is complicated by the existence of two distinct generations, a sexual and an asexual, alternately producing each other. The sex cells are produced by individuals arising from the spores formed at reduction. It is a familiar fact that in the great plant groups the relative extent to which the sexual generation is developed differs very widely. In the thallophytes it may be the only individual; in the bryophytes the gametophyte maintains a strong and independent existence; in the pteridophytes, while the sexual plant is still independent, it is much simplified in structure and reduced in size; in the seed plants the gametophyte becomes a few-celled structure parasitic upon the sporophyte and ordinarily hidden from view.

We might infer from these facts that as one passes from the lower to the higher groups the hereditary potentialities of the respective forms are manifested in a continually decreasing degree in the gametophyte generation. This reaches its culmination in the angiosperms where the gametophyte, while still retaining its essential character, rarely gives any evidence of its inherent genetic organization.

While evidence to prove the point is not available, it is conceivable that among heterozygotes in the lower forms, as a result of differences in development of the sexual individuals incident upon their variable hereditary constitutions, the proportions of the various classes of gametes presented at fertilization might not concur with the ratio in which the corresponding spore types were formed at reduction. We have no reason

to believe that there are not characters in these forms with an extensively developed sexual generation which determine to a greater or lesser extent the degree to which their possessors will share in the process of gametic reproduction. Natural selection may operate on the gametophyte as well as on the sporophyte. As we pass to the higher forms, the opportunity for such modification of zygotic ratios becomes more limited, since the gametophyte is less prominent, and hence, less exposed to the action of any selective forces. It may not be far wrong to conclude that, on the average, the difference in the ratio of the various genetic classes of reproductive cells at sporogenesis as compared with that at fertilization is inversely proportional to the degree to which the gametophyte is reduced.

But the opportunity for selective action between sporogenesis and fertilization has not been wholly eliminated, even in the seed plants. In order that the sperm and egg be brought into apposition the pollen grain must germinate upon the stigma, develop a tube which makes its way through the style to the embryo sac and discharge its contents, including the male nuclei, therein.

It has been known for some time that in self-sterile species a given combination is fertile or sterile, depending upon the ability of the male gametophyte with its complement of gametes to reach the embryo sac. Selective action between pollen tubes having their origin in different sporophytes is here manifested in an extreme degree. JONES (1922) has presented a convincing body of evidence in support of the view that in maize, the plant favors its own pollen when this is in competition with pollen of diverse origin. It is not with such cases as these, however, that the present work is directly concerned, but with differential development among male gametophytes arising from a single individual. The former phenomena involve a reciprocality of action that may require separate consideration.

Soon after the rediscovery of MENDEL'S laws, CORRENS (1902) reported that, in the F_2 generation following a cross between sugary maize and a pop variety, only 15.6 percent of sugary seeds occurred, whereas 25 percent were expected. It was found that when the hybrid was combined reciprocally with the recessive type, the deficiency was not in evidence, indicating that the two sorts of gametes were formed in Mendelian proportions. CORRENS interpreted the anomalous results obtained in F_2 derived from the hybrid by self-pollination as due to some handicap imposed upon the combination of sugary gametes at the time of fertilization. Recently, JONES (1924) has also reported that crosses between certain races of starchy and sugary maize show a significant deficiency of sugary seeds in the F_2 generation and fewer heterozygotes than expected on the Mendelian basis,

when the hybrid is used as the pollen parent in the backcross to the starchy parent. EMERSON (1924) has made an extended study of this case and his findings are available in abstract form. We quote from his summary:

"Crosses of the common varieties of sugar corn with most types of starchy corn give 25 percent of sugary seeds in F_2, with typical Mendelian behavior in later generations and in backcrosses. When, however, rice pop-corn is used as the starchy parent, there results in F_2 a marked deficiency (about 15 percent) of sugary seeds. F_3 progenies from starchy seeds of this cross again throw low-sugar ratios, but with them normal (25 percent) and occasionally high-sugar (35 percent) ratios. In backcross tests, common sugar $\times$ F_1, $F_1 \times$ common sugar, and $F_1 \times$ low-sugar give approximately a fifty-fifty segregation, but low-sugary $\times$ F_1 shows only about 30 percent sugary seeds. Low-sugar strains $\times$ rice pop give 25 percent sugary in F_2, while low-sugary $\times$ other starchy types give high-sugary in F_2. These results are interpreted as due to a factor from rice pop, linked with the starchy-sugary genes, which influences rate of pollen-tube growth, thus resulting in selective fertilization."

This is a very significant series of observations and the details are awaited with interest. It is noteworthy, perhaps, that in this case, where differential pollen-tube growth likewise appears to be the true explanation of the anomalous results, the factor postulated operates in combination with a differential for carbohydrate metabolism occasioned by the starchy-sugary genes.

HERIBERT-NILSSON (1920, 1923) attributes the deficiency of white-nerved individuals following certain combinations in Oenothera, involving the factors *Rr*, which differentiate red and white nerves, to a lower rate of growth of the pollen tubes carrying the recessive *r* factor. CORRENS (1921) has demonstrated that variations in the sex ratio in Melandrium, where the staminate individual is heteromorphic, result from differential development of the two classes of male gametophytes. By means of breeding tests and by direct histological examination, BUCHHOLZ and BLAKESLEE (1922) have shown that the two classes of male gametophytes, the *n* and *n*+1 types, respectively, arising from the Globe mutant of Datura develop at different rates in the style of this form. It seems rather probable that in plants showing irregular chromosome behavior in the reduction divisions, the relatively wide variability in the composition of the spores will lead to differential pollen-tube growth as a regular feature. In such cases the events which transpire between reduction and fertilization may determine to a very considerable extent the character of the succeeding generation. HALLQVIST (1923) shows that the probable cause of the failure of a chlorophyll-deficient dwarf type of barley to appear in Mendelian proportions after certain combinations is restricted development of the pollen tubes bearing the factor concerned. It has been suggested by

BRINK and MACGILLIVRAY (1924) that the deficiency of glutinous grains among the offspring of rice plants heterozygous for this character, as recorded by PARNELL (1921), is due to selective pollen-tube growth.

This brief résumé of the literature shows that the behavior of waxy hybrids in maize does not constitute the only exception in angiosperms to the rule that the distribution of gametes entering into fertilization is uninfluenced by the events which follow reduction. Yet the number of cases in which differential action during this stage has been demonstrated is very small indeed. This, after all, is the fact of greater significance. But why are such exceptions so rare? The male gametophyte, while simple in structure, undergoes a considerable growth and approaches the embryo sac under conditions in which keen competition is not inconceivable. Presumably, the pollen tube during its development is under the control of its nuclei and it might be expected that rate of growth and hence, ability to present gametes at fertilization, would be influenced by its factorial composition. The fact is, however, that differential development of the male gametophyte is very rare. Our studies on the exception afforded by the waxy case have been directed with a view of answering as completely as possible the question as to why this is so.

Chemical investigations in progress in this laboratory should aid materially in solving the problem. The breeding facts reported here, taken in conjunction with the knowledge available from studies on pollen in artificial culture, point, however, to certain tentative conclusions, some of which have been already forecast in the body of this paper.

Insofar as the materials stored in the pollen grain are insufficient for complete development, the male gametophyte of angiosperms is dependent during growth upon the pistillate tissue it traverses. It is clear that an interaction is set up between the pollen tube and the tissue through which it grows, but this mechanism is not well understood. PARNELL'S (1921) work on the glutinous hybrid in rice and the findings in regard to the character of the pollen of maize plants segregating for the waxy character indicate that the metabolic activity of the microgametophyte is controlled by its own nuclei. We are inclined to the view that the mature pollen grain is so organized that the additional requirements for development are, in the main, relatively simple. The cellular organization of the male gametophyte is rudimentary and the duration of its activity, at least in most herbaceous forms, very short. It seems likely, in view of the relatively low plane of differentiation of the pollen tube and the comparative simplicity of its metabolic processes, that few gene differences alter the course of its development. It is only when the factors controlling the activities

of the pollen tube differ significantly in competing individuals that we may expect variations in rate of growth and consequent modification of the zygotic ratio.

The fact is to be noted also that any gene which restricts the development of the male gametophyte will, *ceteris paribus*, tend to eliminate itself from the race. This is an important consideration from the standpoint of Mendelian theory. If nature did not discriminate against such factors, but permitted them to accumulate, the breeding facts, in certain cases, might present quite a different picture than we find; we would be forced to make important reservations in applying the Mendelian concept to seed plants in general. But such genes under competitive conditions must eventually vanish. Their period of survival, other things being equal, is inversely proportional to the disadvantage which they impose upon the gametophytes possessing them. The waxy gene causes but a slight handicap, and, hence, may have persisted for a long time. We might venture the opinion that, if the very irregular results found following crosses of sugary races with Rice pop-corn are due, as EMERSON (1924) contends, to a factor contributed by the starchy parent, this gene does not act as a differential for pollen-tube growth in the Rice Pop variety. This may be due to its being homozygous in that stock or to the absence of other factors necessary to activate it.

This seems to afford a satisfactory explanation of the regularity with which genetic factors are transmitted through the haploid generation of angiosperms without disturbance of the chance ratio.

It would be interesting to know what sort of gene difference would be expected to cause a preferential effect during the development of the male gametophyte. With reference to this point, it may not be without significance that in the two best-known cases of differential pollen-tube growth, namely, in waxy and certain sugary hybrids in maize, genes which occasion modifications in carbohydrate metabolism are involved. We have expressed the opinion that the carbon supply is the most important element in the nutrition of the microgametophyte. If this is true, it is logical to expect that the factors causing selective pollen-tube growth will largely fall within the group concerned in carbohydrate transformations.

SUMMARY

1. Microchemical tests on embryo sacs arising from maize plants heterozygous for waxy show that this gene exerts a differential action in the female gametophyte.

2. Additional evidence is brought forward showing that reciprocal crosses between plants heterozygous for waxy and the homozygous recessive differ in a small but clearly significant way; when the heterozygote is used as the pistillate parent in the combination the proportion of non-waxy and waxy seeds formed is in very close agreement with Mendelian expectation; when, however, individuals heterozygous for the waxy gene are used as pollen parents a significantly smaller proportion of waxy seeds results than is called for on the monohybrid basis.

3. It is demonstrated that this deviation is caused by a lower rate of growth of the pollen tubes carrying the waxy gene.

4. In the progenies examined, the distributions on the ears resulting from the use of pollen produced by plants segregating for the waxy character do not form a homogeneous group. This is taken to indicate that genes other than the non-waxy-waxy pair operate in conjunction with the latter to affect the rate of pollen-tube growth.

5. Preliminary results with one stock suggest the action of a single pair of modifying factors whose effect is to differentiate individuals producing high and low percentages of waxy seeds.

6. Plants homozygous for sugary and heterozygous for waxy were found to produce, on self-pollination, 18.8 percent waxy grains or 6.2 percent less than expected on the Mendelian basis. It would appear that the sugary gene in homozygous condition operates in some manner to increase the difference in rate of growth of non-waxy and waxy pollen tubes.

7. Irradiation of pollen from heterozygous waxy individuals for 15, 30 and 45 minutes with ultra-violet light did not occasion a differential effect.

8. On the other hand, the use of similar pollen desiccated over $CaCl_2$ for 1 and 3 hours at 40°C resulted in the production of a proportion of waxy seeds significantly lower, probably, than that on the control ears.

9. From the phylogenetic point of view the opportunity for selective elimination on account of genetic variations between sporogenesis, where reduction occurs, and fertilization, decreases as we pass from the lower to the higher plants; this reaches its culmination in the angiosperms, but it is pointed out that even in this group the chance of differential action during the period between the reduction divisions and fertilization is not wholly precluded.

10. The general regularity with which genetic factors are transmitted through the male gametophyte in angiosperms is interpreted as being due to (1) the low plane of differentiation and comparative simplicity of the

developmental processes of the pollen tube, presumably affected by relatively few gene differences; (2) the limited opportunity for the establishment in a significant way of such inherent variations in capacity to develop as may exist on account of the transient activity of the gametophyte generation; and (3) the fact that any gene which restricts pollen-tube growth will, other things being equal, tend to eliminate itself from the race.

11. The evidence gleaned from studies of pollen on artificial media and from pedigree-culture experiments indicates that factors causing differential pollen-tube growth are to be looked for, mainly, among those whose chief action relates to carbohydrate transformations.

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APPENDIX—Tables 13 to 17

TABLE 13

Distribution of non-waxy and waxy seeds on single ears resulting from crosses of the type $W_z\ w_z$ ♀ × $w_z\ w_z$ ♂ with progenies R 2a and R 2b.

EAR NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN PERCENT IN WAXY CLASS	DEV. / P.E.
	Total	Non-waxy	Waxy			
1	171	94	77	45.02±2.58	−4.98	1.93
2	286	154	132	46.15±1.99	−3.85	1.93
3	268	140	128	47.76±2.06	−2.24	1.09
4	242	129	113	46.69±2.17	−3.31	1.52
5	238	115	123	51.68±2.19	+1.68	0.77
6	457	228	229	50.10±1.58	+0.10	0.06
7	520	266	254	48.84±1.48	−1.16	0.78
8	336	162	174	51.78±1.84	+1.78	0.97
9	321	168	153	47.66±1.88	−2.34	1.24
10	396	204	192	48.48±1.69	−1.52	0.90
11	476	237	239	50.21±1.55	+0.21	0.13
12	503	254	249	49.50±1.50	−0.50	0.33
13	493	253	240	48.68±1.52	−1.32	0.87
14	319	185	134	42.00±1.89	−8.00	4.23
15	425	216	209	49.17±1.64	−0.83	0.51
16	496	256	240	48.38±1.51	−1.62	1.07
17	593	302	291	49.07±1.38	−0.93	0.67
18	361	187	174	48.19±1.77	−1.81	1.02
19	568	289	279	49.11±1.41	−0.89	0.63
20	566	294	272	48.05±1.42	−1.95	1.37
21	594	273	321	54.04±1.38	+4.04	2.93
22	546	258	288	52.74±1.44	+2.74	1.90
23	661	367	294	44.47±1.31	−5.53	4.22
24	638	321	317	49.68±1.33	−0.32	0.24
25	667	327	340	50.97±1.31	+0.97	0.74
26	436	203	233	53.44±1.61	+3.44	2.14
27	587	319	268	45.65±1.39	−4.35	3.13

TABLE 13 (continued)

EAR NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN PERCENT IN WAXY CLASS	DEV. / P.E.
	Total	Non-waxy	Waxy			
28	550	254	296	53.81±1.44	+3.81	2.64
29	458	202	256	55.89±1.58	+5.89	3.73
30	352	170	182	51.70±1.80	+1.70	0.94
31	370	188	182	49.18±1.75	−0.82	0.47
32	638	315	323	50.62±1.33	+0.62	0.47
33	518	272	246	47.49±1.48	−2.51	1.69
34	451	217	234	51.88±1.59	+1.88	1.18
35	558	258	300	53.76±1.43	+3.76	2.63
36	312	150	162	51.92±1.91	+1.92	1.00
37	332	156	176	53.01±1.85	+3.01	1.63
38	577	306	271	46.96±1.40	−3.04	2.17
39	494	238	256	51.82±1.52	+1.82	1.20
40	463	229	234	50.53±1.57	+0.53	0.34
41	364	176	188	51.64±1.77	+1.64	0.93
42	504	254	250	49.60±1.50	−0.40	0.27
43	357	183	174	48.73±1.78	−1.27	0.71
44	318	164	154	48.42±1.89	−1.58	0.83
45	351	171	180	51.28±1.80	+1.28	0.71
46	301	150	151	50.16±1.94	+0.16	0.08
47	419	207	212	50.59±1.65	+0.59	0.36
48	585	298	287	49.05±1.39	−0.95	0.68
Totals	21,436	10,759	10,677	49.81±0.23	−0.19	0.83

TABLE 14

Distribution of non-waxy and waxy seeds on single ears resulting from crosses of the type $W_x\ w_x \female \times w_x\ w_x \male$ *with progenies R 5b and R 5a.*

EAR NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN PERCENT IN WAXY CLASS	DEV./P.E.
	Total	Non-waxy	Waxy			
1	222	106	116	52.25±2.26	+2.25	1.00
2	341	181	160	46.92±1.83	−3.08	1.68
3	341	182	159	46.62±1.83	−3.38	1.85
4	326	166	160	49.07±1.87	−0.93	0.50
5	303	156	147	48.51±1.94	−1.49	0.77
6	611	323	288	47.13±1.36	−2.87	2.11
7	490	224	266	54.28±1.52	+4.28	2.81
8	484	223	261	53.92±1.53	+3.92	2.56
9	476	270	206	43.27±1.55	−6.73	4.34
10	578	254	324	56.05±1.40	+6.05	4.32
11	449	228	221	49.22±1.59	−0.78	0.49
12	612	300	312	50.98±1.36	+0.98	0.72
13	455	228	227	49.89±1.58	−0.11	0.07
14	613	294	319	52.03±1.36	+2.03	1.49
15	488	260	228	46.72±1.53	−3.28	2.14
16	488	249	239	48.97±1.53	−1.03	0.67
17	405	200	205	50.61±1.68	+0.61	0.36
18	409	208	201	49.14±1.67	−0.86	0.51
19	364	189	175	48.07±1.77	−1.93	1.09
20	365	179	186	50.95±1.76	+0.95	0.54
21	487	242	245	50.30±1.53	+0.30	0.20
22	365	173	192	52.60±1.76	+2.60	1.48
23	497	260	237	47.68±1.51	−2.32	1.54
24	504	244	260	51.58±1.50	+1.58	1.05
25	554	284	270	48.73±1.43	−1.27	0.89
26	514	262	252	49.02±1.49	−0.98	0.66
27	446	238	208	46.63±1.60	−3.37	2.11
28	416	211	205	49.27±1.65	−0.73	0.44
29	604	286	318	52.64±1.37	+2.64	1.93
30	476	250	226	47.47±1.55	−2.53	1.63
31	506	250	256	50.59±1.50	+0.59	0.39
32	512	247	265	51.75±1.49	+1.75	1.17
33	430	218	212	49.30±1.63	−0.70	0.43
34	482	248	234	48.54±1.54	−1.46	0.95
Totals	15,613	7833	7780	49.83±0.27	−0.17	0.63

TABLE 15

Distribution of non-waxy and waxy seeds on single ears resulting from crosses of the type $w_x\ w_x$ ♀ × $W_x\ w_x$ ♂ with progenies R 2b and R 2a.

EAR NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN PERCENT IN WAXY CLASS	DEV. / P.E.
	Total	Non-waxy	Waxy			
1	231	133	98	42.42±2.22	− 7.58	3.41
2	433	244	189	43.64±1.62	− 6.36	3.92
3	316	159	157	49.68±1.90	− 0.32	0.17
4	343	188	155	45.18±1.82	− 4.82	2.65
5	389	190	199	51.15±1.71	+ 1.15	0.67
6	312	157	155	49.67±1.91	− 0.33	0.17
7	459	228	231	50.32±1.57	+ 0.32	0.20
8	388	214	174	44.84±1.71	− 5.16	3.02
9	552	288	264	47.82±1.44	− 2.18	1.51
10	322	176	146	45.34±1.88	− 4.66	2.48
11	544	271	273	50.18±1.45	+ 0.18	0.12
12	524	272	252	48.09±1.47	− 1.91	1.30
13	477	254	223	46.75±1.54	− 3.25	2.11
14	268	151	117	43.65±2.06	− 6.35	3.08
15	432	206	226	52.31±1.62	+ 2.31	1.42
16	380	206	174	45.78±1.73	− 4.22	2.44
17	459	267	192	41.83±1.57	− 8.17	5.20
18	513	255	258	50.29±1.49	+ 0.29	0.19
19	600	333	267	44.50±1.38	− 5.50	3.98
20	696	366	330	47.41±1.28	− 2.59	2.02
21	547	299	248	45.33±1.44	− 4.67	3.24
22	481	261	220	45.73±1.54	− 4.27	2.77
23	586	300	286	48.80±1.39	− 1.20	0.86
24	535	274	261	48.78±1.46	− 1.22	0.83
25	233	98	135	57.93±2.21	+ 7.93	3.59
26	381	189	192	50.39±1.73	+ 0.39	0.22
27	487	224	263	54.00±1.53	+ 4.00	2.61
28	519	197	322	62.04±1.48	+12.04	8.13
29	511	265	246	48.14±1.49	− 1.86	1.25
30	385	202	183	47.53±1.72	− 2.47	1.44
31	444	215	229	51.57±1.60	+ 1.57	0.98
32	571	326	245	42.90±1.41	− 7.10	5.03
33	455	220	235	51.64±1.58	+ 1.64	1.04
Totals	14,773	7628	7145	48.36±0.28	− 1.64	5.85

TABLE 16

Distribution of non-waxy and waxy seeds on single ears resulting from crosses of the type $w_x\ w_x \varphi \times W_x\ w_x \sigma^{\prime}$ with progenies R 5a and R 5b.

EAR NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN PERCENT IN WAXY CLASS	DEV./P.E.
	Total	Non-waxy	Waxy			
1	393	220	173	44.02±1.70	−5.98	3.52
2	456	263	193	42.32±1.58	−7.68	4.86
3	500	263	237	47.40±1.51	−2.60	1.72
4	415	195	220	53.01±1.66	+3.01	1.81
5	357	178	179	50.14±1.78	+0.14	0.08
6	373	172	201	53.88±1.75	+3.88	2.22
7	262	132	130	49.61±2.08	−0.39	0.19
8	440	219	221	50.22±1.61	+0.22	0.14
9	349	197	152	43.55±1.80	−6.45	3.58
10	455	237	218	47.91±1.58	−2.09	1.32
11	468	232	236	50.42±1.56	+0.42	0.27
12	510	209	301	59.01±1.49	+9.01	6.05
13	544	275	269	49.44±1.45	−0.56	0.39
14	518	272	246	47.49±1.48	−2.51	1.69
15	456	258	198	43.42±1.58	−6.58	4.16
16	432	230	202	46.75±1.62	−3.25	2.01
17	521	275	246	47.21±1.48	−2.79	1.88
18	475	236	239	50.31±1.55	+0.31	0.20
19	379	194	185	48.81±1.73	−1.19	0.69
20	349	172	177	50.71±1.80	+0.71	0.39
21	329	155	174	52.88±1.86	+2.88	1.55
22	432	216	216	50.00±1.62	0.00	0.00
23	472	257	215	45.55±1.55	−4.45	2.87
24	390	215	175	44.87±1.71	−5.13	3.00
25	629	316	313	49.76±1.34	−0.24	0.18
26	399	220	179	44.86±1.69	−5.14	3.04
27	495	254	241	48.68±1.52	−1.32	0.87
28	452	243	209	46.23±1.59	−3.77	2.37
29	330	177	153	46.36±1.86	−3.64	1.96
30	301	149	152	50.49±1.94	+0.49	0.25
31	608	309	299	49.17±1.37	−0.83	0.61
32	535	315	220	41.12±1.46	−8.88	6.08
33	519	275	244	47.01±1.48	−2.99	2.02
34	367	181	186	50.68±1.76	+0.68	0.39
35	265	119	146	55.09±2.07	+5.09	2.46
36	552	308	244	44.20±1.44	−5.80	4.03
37	388	189	199	51.28±1.71	+1.28	0.75
38	408	216	192	47.05±1.67	−2.95	1.77
39	481	246	235	48.85±1.54	−1.15	0.75
40	509	265	244	47.93±1.49	−2.07	1.39
41	392	222	170	43.36±1.70	−6.64	3.90
42	342	182	160	46.78±1.82	−3.22	1.77
Totals	18,247	9458	8789	48.17±0.25	−1.83	7.32

TABLE 17

Distribution of non-waxy and waxy seeds on single ears resulting from self-pollination of $s_u\ s_u\ W_x\ w_x$ plants in progeny R 11.

EAR NUMBER	NUMBER OF SEEDS			PERCENT WAXY	DEVIATION IN PERCENT IN WAXY CLASS	DEV. / P.E.
	Total	Non-waxy	Waxy			
1	509	427	82	16.11±1.29	− 8.89	6.89
5	430	348	82	19.06±1.41	− 5.94	4.21
11	320	265	55	17.18±1.63	− 7.82	4.80
12 (a)	474	397	77	16.24±1.34	− 8.76	6.54
12 (b)	293	236	57	19.45±1.71	− 5.55	3.24
13	320	262	58	18.12±1.63	− 6.88	4.22
14	149	122	27	18.12±2.39	− 6.88	2.88
15	238	196	42	17.64±1.89	− 7.36	3.89
22	569	453	116	20.38±1.22	− 4.62	3.79
23 (a)	188	154	34	18.08±2.13	− 6.92	3.25
23 (b)	234	190	44	18.80±1.91	− 6.20	3.25
24	571	457	114	19.96±1.22	− 5.04	4.13
26	379	317	62	16.35±1.50	− 8.65	5.77
27	445	370	75	16.85±1.38	− 8.15	5.90
32	425	348	77	18.11±1.41	− 6.89	4.89
33	343	277	66	19.24±1.58	− 5.76	3.64
34	285	231	54	18.94±1.73	− 6.06	3.50
36	538	445	93	17.28±1.26	− 7.72	6.13
37	444	366	78	17.56±1.39	− 7.44	5.35
39	181	154	27	14.91±2.17	−10.09	4.65
45	647	528	119	18.39±1.14	− 6.61	5.80
46	409	309	100	24.44±1.44	− 0.56	0.39
47	611	510	101	16.53±1.18	− 8.47	7.18
49 (a)	288	222	66	22.91±1.72	− 2.09	1.21
49 (b)	371	302	69	18.59±1.52	− 6.41	4.22
50 (a)	374	297	77	20.58±1.51	− 4.42	2.93
50 (b)	666	504	162	24.32±1.13	− 0.68	0.60
54 (a)	204	161	43	21.07±2.04	− 3.93	1.93
54 (b)	530	427	103	19.43±1.27	− 5.57	4.38
63 (a)	272	232	40	14.70±1.77	−10.30	5.82
63 (b)	557	446	111	19.92±1.24	− 5.08	4.10
Totals	12,264	9953	2311	18.84±0.26	− 6.16	23.69

LINKAGE BETWEEN SWEET-DEFECTIVE AND SUGARY ENDOSPERM IN MAIZE

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	395
Additional data on the inheritance of sweet-defective	395
Linkage between defective endosperm and sugary endosperm	397
SUMMARY	401
LITERATURE CITED	401

INTRODUCTION

In the spring of 1922 Doctor E. W. LINDSTROM, then at the UNIVERSITY OF WISCONSIN, sent the writer some normal seed from a self-pollinated ear of Golden Bantam sweet-corn which was segregating for a very distinct type of defective endosperm. LINDSTROM (1923) has since published data which show that this character is inherited as a simple Mendelian recessive.

During the summer of 1922 a number of crosses were made between this Golden Bantam strain and five different starchy strains. Ears resulting from these crosses were planted in the spring of 1923 and a large number of selfings made. The data presented in this paper are from these self-pollinated ears.

ADDITIONAL DATA ON THE INHERITANCE OF SWEET-DEFECTIVE

From the original seed furnished by LINDSTROM, twenty-one plants were grown to maturity. Of these twenty-one plants, sixteen were either selfed or used in crosses which were selfed in the next generation. Of the sixteen plants, twelve proved to be segregating for defective endosperm, giving a ratio of 4 pure normal to 12 segregating ears, as compared with a theoretical ratio of 5.33 to 10.67, assuming a simple monohybrid condition.

In the spring of 1923 seed was planted from ten different crosses. All of these crosses happened to be with pure starchy endosperm plants either as female or male parents. Of these crosses all segregated for sugary endosperm in the F_2 generation as expected, and seven segregated for defective endosperm as well as the sugary character. From the seven

crosses segregating for defective endosperm, a total of 160 selfed ears was obtained. Of these 160 selfed ears, 91 were segregating for defective endosperm. This gives a ratio of sixty-nine homozygous normal to ninety-one segregating ears, as compared with a theoretical ratio of 80 : 80.

Each of the 91 ears was shelled and the number of normal and defective kernels counted. They gave a total of 30,952 kernels, of which 23,222 were normal and 7730 defective, as compared with a theoretical ratio of 23,214 to 7738, based on a simple monohybrid condition. The data from the selfed ears are presented in table 1, by rows.[1] Rows 37 and 38 are from the same cross and the same is true of rows 109 and 110. In each of these two cases the two rows were planted from different-colored seeds on the same ear. Rows 94 and 194, and 137 and 191 are reciprocal crosses.

TABLE 1

Data on normal and defective endosperm.

ROW NUMBER	NUMBER OF SEGREGATING EARS	NORMAL SEEDS	DEFECTIVE SEEDS	TOTAL	DEVIATION FROM 3 TO 1 RATIO		
					DEVIATION	P.E.	$\frac{\text{DEV.}}{\text{P.E.}}$
37	14	3290	1081	4371	11.75	19.31	0.61
38	7	1774	646	2420	41.00	14.36	2.86
53	11	3160	1089	4249	26.75	19.04	1.40
74	8	2014	701	2715	22.25	15.21	1.46
85	8	2147	529	2676	140.00	15.11	9.27
94	10	2283	792	3075	21.25	16.20	1.31
109	4	917	265	1182	30.50	10.04	3.04
110	8	2228	801	3029	43.75	16.08	2.72
137	8	2015	711	2726	29.50	15.25	1.93
191	11	3010	1002	4012	1.00	18.50	0.05
194	2	384	113	497	11.25	6.51	1.73
Total	91	23,222	7730	30,952	8.00	51.35	0.16
Not including No. 85	83	21,075	7201	28,276	132.00	49.08	2.69

One very wide deviation from theoretical expectation is seen in row 85. This row exhibits such a wide deviation from the expected 3 : 1 ratio that it obviously should be considered separately. A possible reason for this behavior will be discussed later in the paper.

It so happens that the wide deviation in this row makes the deviation from expectation in the grand total smaller than it should be if this row

[1] Data for individual ears are available.

had behaved in the normal way. Eliminating row 85 from the data, the deviation divided by the probable error of the total is 2.69. In most of the rows where the deviations were rather large there were too many defective seeds. These deviations are probably due largely to improper classification caused by the presence of other defective types in some of the ears. In order to give some idea of the amount of deviation of individual ears from the theoretical 3 : 1 ratio, the maximum and minimum deviations of individual ears within each row, expressed by the deviation divided by the probable error, are presented in table 2. Except for row 85, none of the deviations are unreasonably large considering the type of character studied.

TABLE 2

Maximum and minimum deviations of individual ears in each row from a 3 : 1 ratio of normal to defective endosperm.

ROW NUMBER	NUMBER OF SEGREGATING EARS	DEVIATION DIVIDED BY PROBABLE ERROR	
		Maximum	Minimum
37	14	3.52	0.18
38	7	3.95	0.74
53	11	3.50	0.00
74	8	1.55	0.09
85	8	7.05	0.40
94	10	3.00	0.49
109	4	4.05	0.62
110	8	3.63	0.92
137	8	3.07	0.79
191	11	2.59	0.43
194	2	2.43	0.05

LINKAGE BETWEEN DEFECTIVE ENDOSPERM AND SUGARY ENDOSPERM

When the ears segregating for both defective and sugary endosperm were harvested, it was noticed at once that there were far too few normal sugary seeds present. This suggested a linkage between defective and sugary endosperm, although it was impossible to tell which of the defective seeds were sugary and which were starchy. It was necessary, therefore, to study the material on the basis of a 9 : 3 : 4 phenotypic ratio.

In table 3 the observed data on the three classes of seeds are compared with what would be expected on the basis of independent inheritance. It may be seen at a glance that the observed numbers deviate so far from the expected 9 : 3 : 4 ratio that independent inheritance can not be assumed.

TABLE 3

Comparison of observed and expected numbers of seeds on the basis of independent inheritance of sugary and defective endosperm.

ROW NUMBER	$S_u D_e$		$s_u D_e$		$S_u d_e + s_u d_e$	
	Observed	Expected	Observed	Expected	Observed	Expected
37	3222	2458.7	68	819.6	1081	1092.8
38	1730	1361.2	44	453.7	646	605.0
53	3083	2390.0	77	796.7	1089	1062.2
74	1927	1527.2	87	509.1	701	678.8
85	2117	1505.2	30	501.7	529	669.0
94	2265	1729.7	18	576.6	792	768.8
109	903	664.8	14	221.6	265	295.5
110	2188	1703.8	40	567.9	801	757.2
137	1992	1533.3	23	511.1	711	681.5
191	2932	2256.7	78	752.2	1002	1003.0
194	380	279.5	4	93.2	113	124.2
Total	22,739	17,410.5	483	5803.5	7730	7738.0
Not including No. 85	20,622	15,905.3	453	5301.8	7201	7069.0

The data in table 3 suggest a very close linkage between defective and sugary endosperm. Since it is impossible to make backcrosses on pure-defective plants it is necessary to determine the gametic ratios from the F_2 phenotypic ratios. CASTLE (1916) has published a table giving gametic ratios for different F_2 zygotic ratios. By comparing the data in table 3 with a table made up in the same manner as CASTLE'S it was determined by inspection that the F_2 phenotypic ratio at hand fits the gametic ratio of 30 : 1 closer than any other. This is a crossover percentage of 3.23. Table 4 shows the actual numbers observed in the F_2 and the numbers expected on the basis of a 30 : 1 gametic ratio, not including data from row 85.

TABLE 4

Comparison of observed and expected numbers on the basis of a gametic ratio of 30 : 1.

	$S_u D_e$	$s_u D_e$	$S_u d_e + s_u d_e$
Observed.....	20,622.0	453.0	7201.0
Expected.....	*20,758.3*	*448.7*	*7069.0*
Deviation.....	−136.3	+4.3	+132.0

$X^2 = 3.40100$ $P = .188012$

The fit of the observed with the calculated numbers in table 4 is only fair. That this is due to the excess of defective seeds previously mentioned, is seen from table 5 where the theoretical numbers of sugary and starchy seeds are calculated disregarding the defective seeds.

TABLE 5

Comparison of observed and expected numbers of starchy and sugary seeds where the defective seeds are disregarded.

	$S_u D_e$	$s_u D_e$
Observed.....	20,622.0	453.0
Expected.....	*20,629.1*	*445.9*
Deviation.....	−7.1	+7.1

This very close fit does not prove, however, that there is linkage between sugary and defective endosperm. There could have been some other factor causing the small number of normal sugary seeds. Fortunately, though, as previously mentioned, there were 69 ears in these same rows which were not segregating for defective seeds. If the low number of

TABLE 6

Data on starchy and sugary endosperm, from ears not segregating for defective endosperm.

ROW NUMBER	NUMBER OF SEGREGATING EARS	STARCHY SEEDS	SUGARY SEEDS	TOTAL	DEVIATION FROM 3 TO 1 RATIO		
					DEVIATION	PROBABLE ERROR	DEV. / P.E.
37	5	1446	462	1908	15.00	12.73	1.28
38	9	2194	721	2915	7.75	15.74	0.49
53	6	1641	560	2201	9.75	13.70	0.71
74	11	2605	903	3508	26.00	17.31	1.50
85	7	1780	316	2096	208.00	13.39	15.53
94	7	1764	600	2364	9.00	14.19	0.63
109	8	2173	741	2914	12.50	15.74	0.79
110	3	708	241	949	3.75	9.00	0.42
137	9	2139	712	2851	0.75	15.60	0.05
191	0	....	...	....	.....	.....	....
194	4	719	235	954	3.50	9.02	0.39
Total	69	17,069	5491	22,560	149.00	43.86	3.40
Not including No. 85	62	15,289	5175	20,464	59.00	41.76	1.41

normal sugary seeds had been due to some factor other than the factor causing defective seeds, then one would expect to find this same deficiency of sugary seeds in these ears. The 69 ears were shelled and counted for starchy and sugary seeds and the data tabulated in tables 6 and 7. The number of sugary seeds shown by these tables gives, with the exception of row 85, very close approximation to the normal 3 : 1 ratio; thus, we have rather conclusive proof that the small number of normal sugary seeds on the ears segregating for defective must have been due to a close linkage of sugary and defective seeds.

TABLE 7

Maximum and minimum deviations of individual ears in each row, for sugary endosperm.

ROW NUMBERS	NUMBER OF SEGREGATING EARS	DEVIATION DIVIDED BY PROBABLE ERROR	
		Maximum	Minimum
37	5	1.80	0.31
38	9	2.90	0.04
53	6	2.54	0.23
74	11	3.36	0.35
85	7	8.42	2.33
94	7	2.45	0.23
109	8	2.20	0.09
110	3	1.73	0.54
137	9	2.43	0.39
191	0	....	....
194	4	1.94	0.06

It will be recalled that row 85 in table 1 showed a marked deficiency of defective seeds when compared with the numbers expected on the basis of a 3 : 1 ratio. Of the seven selfed ears in this row not segregating for defective seeds, only one had approximately the expected 25 percent of defective seeds. The other six ears showed percentages ranging from 8.2 to 16.4. Evidently the parent of this row was heterozygous for some factor besides the one causing defective seeds,[2] which greatly reduced the number of sugary seeds, and, since it has been shown that defective seeds is linked with sugary endosperm, the percentage of defective seeds would also be reduced.

[2] Unpublished data of Professor R. A. EMERSON show that when sugary endosperm is crossed with Rice pop-corn the number of sugary endosperm seeds in the F_2 falls markedly short of the expected 25 percent. The pedigree of row 85 was studied and was found to trace back through several generations to one of Professor EMERSON'S crosses involving Rice pop-corn.

SUMMARY

1. Additional data are presented on the inheritance of LINDSTROM's sweet-defective endosperm in Golden Bantam sweet corn.

2. Linkage between this defective endosperm type and sugary endosperm is reported, with a crossover percentage of 3.2.

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